DECLARATION 1

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment or both, under § 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the subject application or any patent issued thereon.

This Declaration is being made in order to distinguish the claimed invention from the teachings of the references Hollingsbee et al. (WO 97/02845) and Tsubouchi et al. (US 6,175,053) cited in the claim rejections according to § 103.

1. The subject-matter of the objected patent Tsubouchi is a wound dressing that is based on a protein material (fibroin and sericitin) that is fully biocompatible and has a very good water absorption properties which results in the material not adhering to the wound. Various additives (disinfectants and others) are added to this basic material. The examples of disinfectants include, among others, iodine, potassium iodide, hydrogen peroxide and povidon iodine. However, it is to be noted that it is not clearly described what exactly was used as a disinfectant in case of a film. Iodine and potassium iodide are presented separately, separated by a comma. The agent that may be used as a disinfectant is actually a solution of iodine in potassium iodide (not iodine or potassium iodide separately). The mixture of iodine and potassium iodide in a solution gives rise to potassium triiodide which releases elementary iodine that is the very disinfectant. If iodine and potassium iodide were used separately then potassium iodide has no disinfecting effects, and iodine does not dissolve in the concentration that the presence of a disinfectant is presented (e.g. column 3, lines 9-12); the claims do not mention the amount of disinfectants at all. It is most likely that the author of the patent included the above mentioned disinfectants without even trying whether it is possible to prepare such a film.

   Even if we admitted that the author of the patent did mean the solution of iodine in potassium iodide, the truth is that it is impossible to prepare a film containing iodine, resp. iodine with potassium iodide as a disinfectant in an equilibrum by means of the processes described in the patent. The substance of the problem lies in that the preparation of the film requires its drying. The description discloses (e.g. column 7, lines 60 – 67; column 9, line 46 – column 10, line 3) that the solution of proteins the film was being prepared of was, together with the disinfectant, cast on a horizontal acrylic resin plate and dried at room temperature. If said solution contained the solution of iodine in potassium iodine, then one must realize that the triiodide does not exist in the solid state, it decomposes to iodine and potassium iodide while iodine gradually evaporates at the film drying conditions and thus moves the equilibrum that exists in the solution between iodine, iodide and triiodide (iodine + iodide → triiodide) towards the triiodide decomposition. This leads to an effect where there is no iodine in the film within a certain time, resp. if iodine is present then just in a trace amount that is not able to exhibit disinfecting properties. Regarding the fact that we have examined the question of stability of iodine in a potassium iodide solution very thoroughly, we have the information on the rate of iodine volatilization – please see graph 1 (Enclosure I) showing the volatilization

of iodine from the solution of iodine in potassium iodide which is enclosed; it can be clearly seen therefrom that in 24 hours there is only 20 % of the initial amount of iodine in the solution. That means that iodine could not be present as a disinfectant in the film. In such a case a question arises as to whether a patent disclosing something unable to be prepared and even contradicting the physical laws may be objected as a state of the art. Moreover, please note the paragraph [0026], of US 2005/0181025 A1, with an explanation of why iodine alone cannot be used in a solution.

The same applies to hydrogen peroxide that is stated as a disinfectant in a dry film as well. According to our opinion, hydrogen peroxide does not exist in a dry film and if there were some traces thereof, it would be improper to call it a disinfectant. We believe that the author of the patent had neither examined said substances as disinfectants nor proved that the film containing them really had disinfecting effects, actually we suppose he just simply included them into the description.

Example 4 of Tsubouchi (US 6,175,053, column 13) describes a preparation of a film containing povidon iodine. Povidon iodine is an organic compound which is totally different from an inorganic complex potassium triiodide, and the fact that both contain iodine atoms does not imply that they might be classified as similar compounds. Povidon iodine has different biological effects in the mixture with hyaluronate and thus, cannot be freely interchanged with other iodine compounds.

Now, as far as the difference between the Tsubouchi document and our invention is concerned, it lies in that Tsubouchi discloses the use of a wound dressing material containing fibroin and sericitin – there is no mention of hyaluronic acid. In our case, while examining the interaction of iodine and potassium triiodide with various polysaccharides, we found out that the hyaluronate solution is partly stabilized by iodine in the solution and forms mixtures therewith that have a totally unexpected favorable effect on wound healing. It showed up that the other polysaccharides tested (6 in total), not even oxycellulose the structure of which is similar to the one of hyaluronate, do not have similar properties to those of hyaluronate in the mixture with iodine complex. The disinfecting properties of iodine complex were just advantageously used in the hyaluronate stabilization. The hyaluronate stabilization lies in its ability to inhibit bacteria that decompose the hyaluronate very fast. Iodine and potassium iodide form potassium triiodide in a solution which is unstable as well due to the volatility of iodine but when using a diluted solution of iodine and potassium iodide, the equilibrum is moved towards the free iodine. Therefore, the diluted potassium triiodide makes the hyaluronate stable and thus the wound healing properties thereof may be exerted. Moreover, a synergistic effect of this mixture was proved: a mixture of hyaluronate with potassium triiodide healed the wound much faster than the hyaluronate without potassium triiodide (see graph 2 – Enclosure II, and fig. 1 – Enclosure III). Potassium triiodide itself, without hyaluronate, showed a tendency to act in a rather inhibitory manner, it did not positively influence the wound healing at all. Moreover, our studies showed that the use of hyaluronate with potassium triiodide enhances the growth of granulation tissue forming in the wound and results in an increase of glycosaminoglycanes amount in this granulation tissue (see [0025] and [0026] of US 2005/0181025 A1). It is to be noted that the granulation tissue formation is the second, very important step in the wound healing. Therefore, the acceleration of the granulation tissue formation leads to an acceleration of the wound healing. Neither the hyaluronate alone, nor the potassium triiodide complex alone are able to produce an increased amount of the granulation tissue (see graphs 2a-b, Enclosure IV).

2. Hollingsbee et al. teach compositions of hyaluronic acid having molecular weights between 50,000 to 2,000,000 and they teach that the hyaluronic acid can be in a salt form, specifically sodium salt, and that antimicrobial agents may be added to hyaluronic acid, such as polyvinylpyrrolidone iodine. The iodine – potassium iodide complex is not mentioned at all because iodine would volatilize from the film (see the explanation above and [0026] of US 2005/0181025 A1). The Hollingsbee's mixture of hyaluronate with povidon-iodine does not exhibit any positive effects on wound healing that could be comparable to the effects of the mixture of hyaluronate with iodine + potassium iodide complex. In Hollingsbee, there is no mention of any positive effect of the mixture of hyaluronate with povidon-iodine on the rate of wound healing, on the granulation tissue formation and the like. Neither does the technical literature mention any effects of the mixture of hyaluronate with povidon-iodine similar to those of a combination of hyaluronate with iodine + potassium iodide complex. Moreover, according to the results of the tests that we performed in developing a new wound-healing preparative, povidon-iodine in the mixture with hyaluronate showed no exceptional effect on wound healing such as the solution of iodine in iodide did. Povidon-iodine is considered to be a disinfectant only which is not the case of the solution of iodine in potassium iodide the main function of which is hyaluronate stabilization (see the explanation above). Povidon iodine is a totally different compound, has different biological effects in the mixture with hyaluronate and thus, cannot be freely interchanged with iodine compounds.

   Further, we would like to point out that the patent document WO 97/02845 of Hollingsbee was objected in the international search report and was classified as an "A" document, i.e. a document defining the general state of the art which is not considered to be of particular relevance (see Enclosure V).

3. In the Office Action, p. 5-6, it is objected that since potassium (or sodium) iodide and iodine are both extremely well known in the art for their disinfecting ability, it would have been obvious to one of ordinary skill in the art at the time of the invention to use said solution as a disinfectant for treating wounds. A real person skilled in the art of wound healing knows that the solution of iodine in potassium (or sodium) iodide had been used for wound disinfection in the 19th century and in the beginning of the 20th century, i.e. in a time when no other disinfecting preparative having the same or similar efficiency had been known. Early after its introduction to use it showed up that such a solution is very unstable, exhibits very irritable properties to the wound, its application causes pain to the patients, it doesn't support the wound healing and, moreover, it colors the surrounding skin and textile that it comes into contact with very strongly [see Enclosure VI: R.A.Cooper: Iodine revisited, Int Wound J 4, 124-137 (2007)]. Efforts to substitute the solution of iodine in potassium iodide by another disinfectant that would be more suitable for wound healing had been made a hundred years ago[see Enclosure VII: H.D.Dakin: On the use of certain antiseptic substances in the treatment of infected wounds, Brit J Med 318-320 (1915)]. The attempts to replace the solution of iodine in iodide by another agent had continued until 1949 when the first practically utilizable iodoform on the basis of polyvinylpyrrolidone which has been used since then was discovered. The doctors' opinion of the solution of iodine in iodide is clearly expressed in the article by F.C. Kelly in which he says: "END OF AN ERA. Last and perhaps greatest protagonist of direct energetic iodine disinfection of wounds was Sir LEONARD ERSKINE HILL, F.R.S. (1866-1952) director of research, St. John Clinic and Institute of Physical Medicine, London. Convinced iodine veteran of World War I, he returned to its defence on outbreak of World War II, extolling virtues of intensive iodine disinfection of wounds 'in all their depths and ramifications'. Views not accepted by the moderns. With

him died finally the 1914-1918 war faith in the iodine 'first field dressing'." [see Enclosure VIII: F.C.Kelly: Iodine in medicine and pharmacy. Since its discovery – 1811 – 1961, Proc Royal Soc Med, 54, 831-836 (1961)]. After the discovery of the complex of polyvinylpyrrolidone with iodine (povidon iodine, PVP-iodine) the use of the solution of iodine in iodide ceased because it was replaced by a much better preparative that eliminated all of the above mentioned negative features of the solution of iodine in iodide and, moreover, it proved itself 3x safer than the solution of iodine in iodide [see Enclosure IX: H.A. Shelanski, M.V. Shelanski: PVP-iodine: history, toxicity and therapeutic uses, J Internat Coll Surgeons, 25, 727-734 (1956)]. Therefore, in view of all of the above mentioned facts, the solution of iodine in iodide had to be used as a disinfectant to wounds in spite of its adverse effects only until a substitute for this solution was found which had no adverse effects and, moreover, was less toxic and thus safer. We are convinced that nowadays, when a broad spectrum of antibiotics, cationic antimicrobial agents, antimicrobial peptides and many other newer, safer and more effective disinfectants are available, a real expert in this subject field would hardly revert to the solution of iodine in iodide as a disinfectant. Firstly, he would have to face the problems of negative organism reactions to the preparative, secondly, he would have to deal with the higher toxicity and further, he would have to eliminate the lack of stability of the solution. If someone reverts to the solution of iodine in iodide, it is for other reasons than its disinfecting ability, such as we did it.

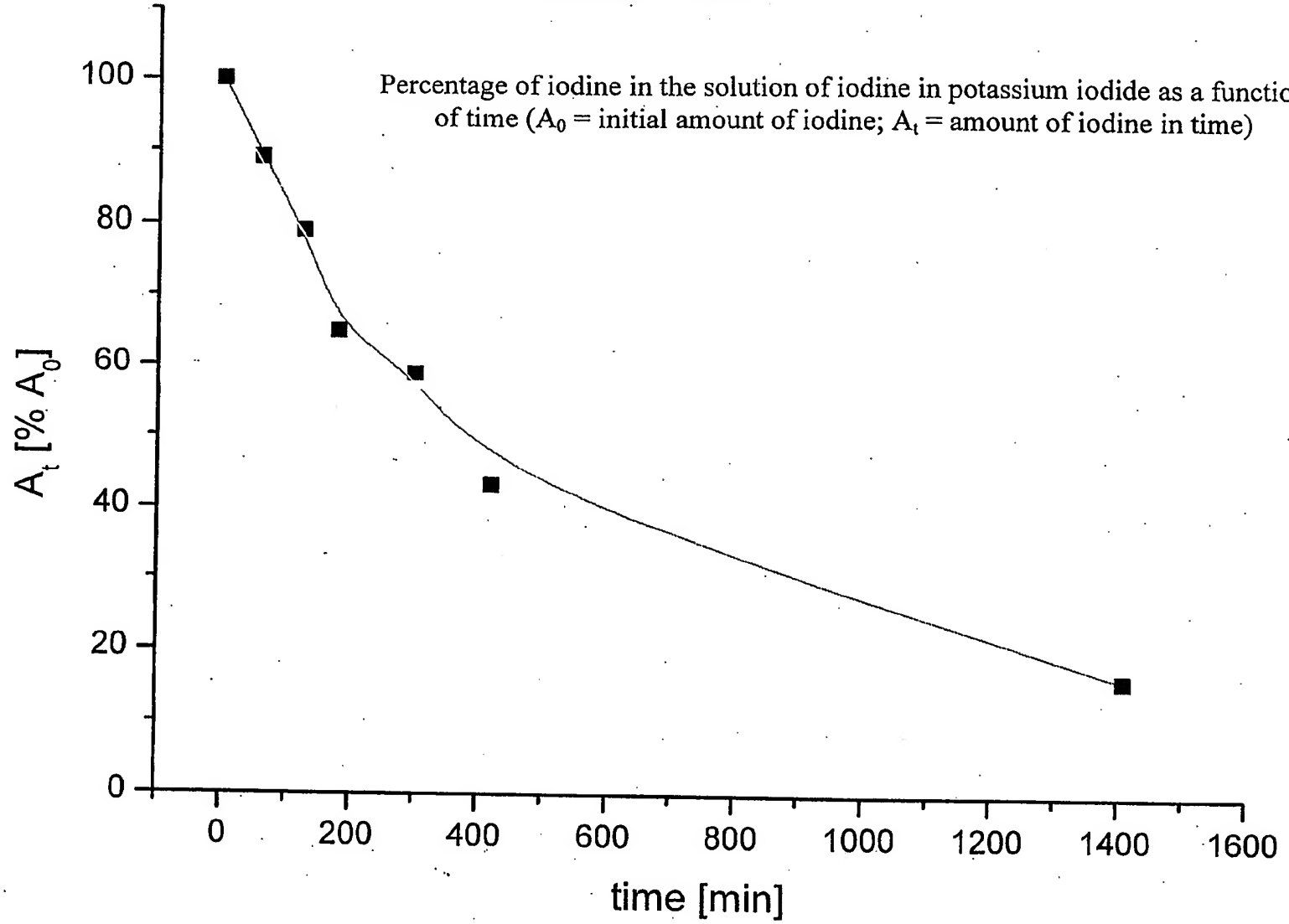
Moreover, the fact that no patent application (except of ours) claiming a use of a mixture of hyaluronate with a solution of iodine in iodide has been filed up till now clearly shows that a mixture of hyaluronate with a solution of iodine in iodide is not something that would occur to a person skilled in the art and that simple mixing of said substances would suffice.

ENCLOSURE I

Graph 1

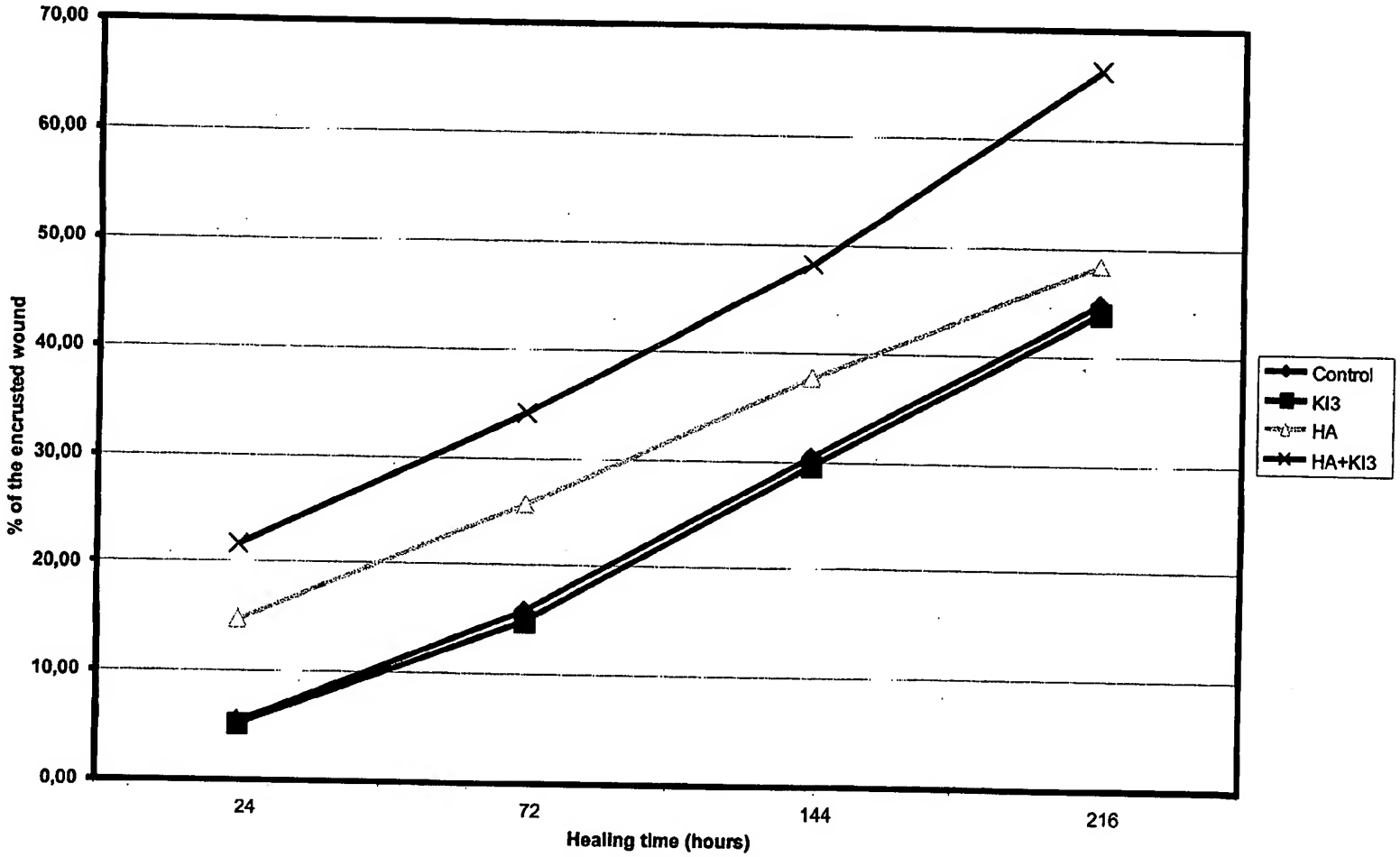
Volatility of Iodine

Percentage of iodine in the solution of iodine in potassium iodide as a function of time (A_0 = initial amount of iodine; A_t = amount of iodine in time)



ENCLOSURE II

Graph 2

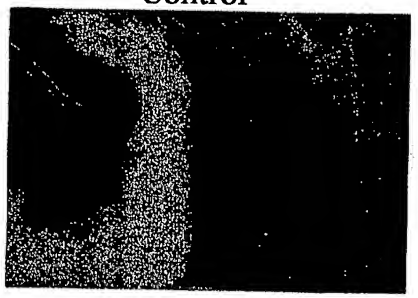


ENCLOSURE III

Figure 1

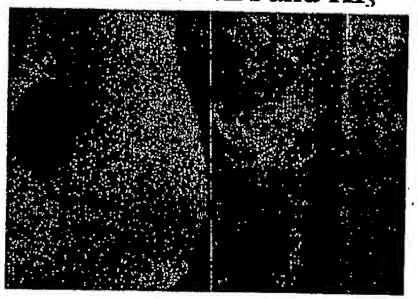
Newly developed bandage for wound healing on the polymer basis

Control



Hyaluronate

Mixture of HA and KI_3



ENCLOSURE IV

Graph 2a

Explanation

Crust = exudate + granulation tissue + the remaining healing solution after the application

FYZ = physiological solution

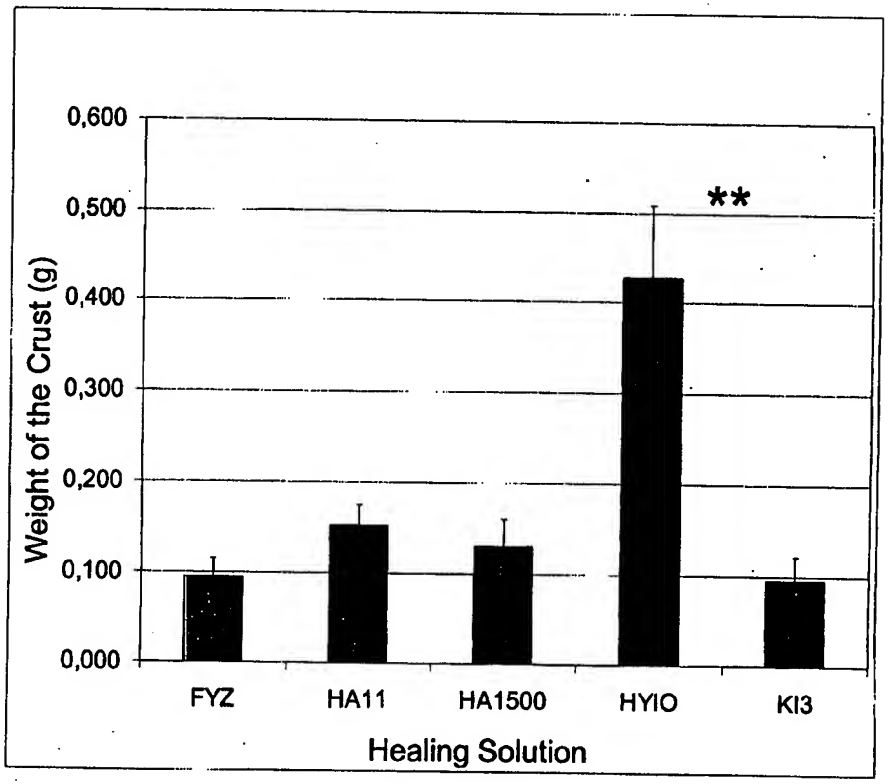
HA11 = hyaluronate having the weight average molecular weight of 11 kDa

HA1500 = hyaluronate having the weight average molecular weight of 1500 kDa

HYIO = Hyiodine, i.e. the mixture of hyaluronate with potassium triiodide complex

KI3 = potassium triiodide complex

Number of rats tested n = 15



Explanation

UK = uronic acid (the total amount of uronic acids in the crust in g is presented on the y axis)

Crust = exudate + granulation tissue + the remaining healing solution after the application

FYZ = physiological solution

HA11 = hyaluronate having the weight average molecular weight of 11 kDa

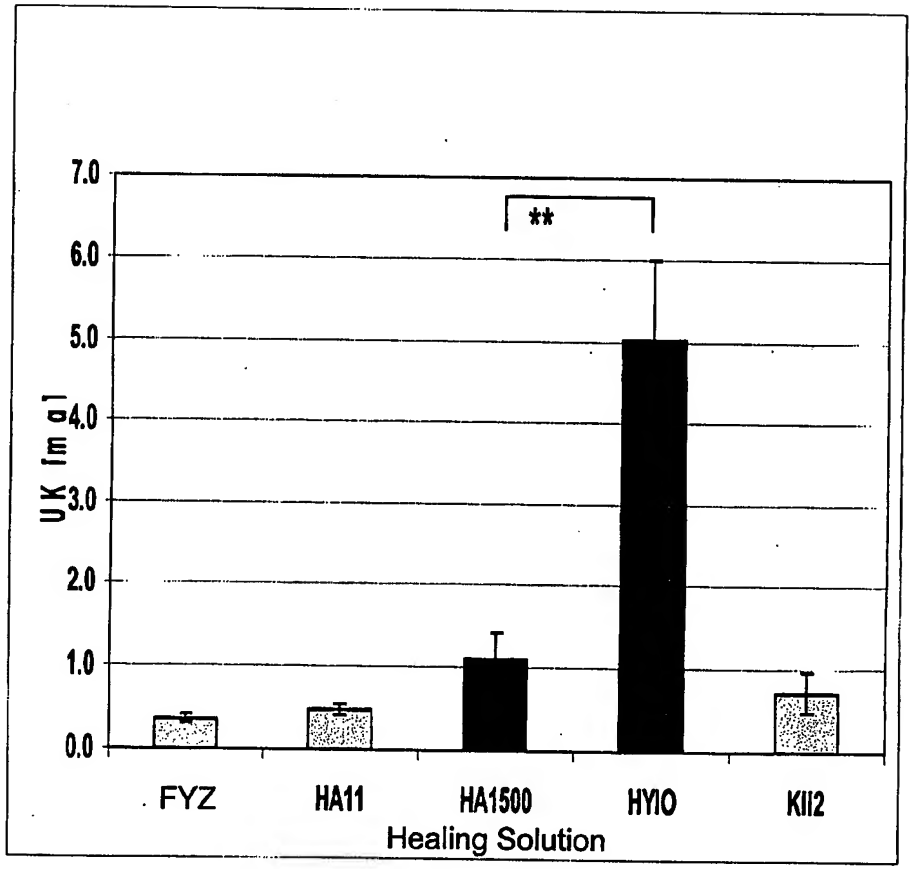
HA1500 = hyaluronate having the weight average molecular weight of 1500 kDa

HYIO = Hyiodine, i.e. the mixture of hyaluronate with potassium triiodide complex

KI3 = potassium triiodide complex

Number of rats tested n = 13

Graph 2b



(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
24 July 2003 (24.07.2003)

PCT

(10) International Publication Number
WO 2003/059404 A3

(51) International Patent Classification[7]: A61L 15/28, 26/00

(21) International Application Number: PCT/CZ2003/000003

(22) International Filing Date: 15 January 2003 (15.01.2003)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
PUV02-12746 18 January 2002 (18.01.2002) CZ

(71) Applicant *(for all designated States except US)*: CPN SPOL. S R.O. [—/CZ]; Tvardkova 1191, 562 01 Usti nad Orlici (CZ).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: VELEBNY, Vladimir [CZ/CZ]; V lukach 1370, 56201 Usti nad Orlici (CZ). SOBOTKA, Lubos [CZ/CZ]; K osade 730, 500 02 Kralove Hradec (CZ). PAVEK, Stanislav [CZ/CZ]; Horni Soupnice 191, 565 53 Sloupnice (CZ). RUZICKOVA, Jana [CZ/CZ]; Moravska 2718, 767 01 Kromeriz (CZ).

(74) Agent: MATUSKOVA, Martina; Mendlove nam. 1a, 603 00 Brno (CZ).

(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, SE, SI, SK, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:
— *with international search report*

(88) Date of publication of the international search report: 18 March 2004

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 2003/059404 A3

(54) Title: PREPARATION FOR WOUND HEALING AND PREVENTION OF BANDAGE ADHESION TO THE WOUND

(57) Abstract: Preparation for wound healing and prevention of adhesion to the wound containing a physiologically acceptable salt of hyaluronic acid, iodine and potassium iodine.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/CZ 03/00003

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A61L15/28 A61L26/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 A61L

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
WPI Data, EPO-Internal, PAJ, CHEM ABS Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	DATABASE WPI Section Ch, Week 199910 Derwent Publications Ltd., London, GB; Class A96, AN 1999-114793 XP002245695 & JP 10 338638 A (TOA YAKUHIN KK), 22 December 1998 (1998-12-22) abstract	1-5
A	WO 97 02845 A (SQUIBB BRISTOL MYERS CO ;HOLLINGSBEE DEREK (GB); JACQUES ELIZABETH) 30 January 1997 (1997-01-30) claims; examples	1-6
A	US 5 442 053 A (DELLA VALLE FRANCESCO ET AL) 15 August 1995 (1995-08-15) claims	1-6

☐ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
27 June 2003	11/07/2003

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	ESPINOSA, M

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/CZ 03/00003

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
JP 10338638 A	22-12-1998	NONE	
WO 9702845 A	30-01-1997	AU 6698996 A	10-02-1997
		WO 9702845 A1	30-01-1997
		GB 2319729 A ,B	03-06-1998
US 5442053 A	15-08-1995	IT 1212892 B	30-11-1989
		IT 1178041 B	03-09-1987
		IT 1229075 B	17-07-1991
		US 4593091 A	03-06-1986
		US 5631241 A	20-05-1997
		AR 231992 A1	30-04-1985
		AT 54921 T	15-08-1990
		AU 575861 B2	11-08-1988
		AU 3414884 A	18-04-1985
		BE 900810 A2	11-04-1985
		CA 1205031 A1	27-05-1986
		CH 666897 A5	31-08-1988
		DE 3482812 D1	30-08-1990
		DK 485384 A	12-04-1985
		EP 0138572 A2	24-04-1985
		ES 8507573 A1	16-12-1985
		FI 843990 A ,B,	12-04-1985
		FR 2553099 A1	12-04-1985
		HK 66091 A	30-08-1991
		HU 36834 A2	28-10-1985
		IE 57931 B1	19-05-1993
		IL 73217 A	30-06-1991
		IN 163192 A1	20-08-1988
		JP 2611159 B2	21-05-1997
		JP 8259604 A	08-10-1996
		JP 6008323 B	02-02-1994
		JP 61028503 A	08-02-1986
		KR 8601148 B1	18-08-1986
		LU 85582 A1	04-06-1985
		NO 844054 A ,B,	12-04-1985
		PT 79339 A ,B	01-11-1984
		SG 59491 G	23-08-1991
		US 5166331 A	24-11-1992
		US 5925626 A	20-07-1999
		ZA 8407942 A	29-05-1985
		IL 96943 A	15-03-1993
		NZ 209850 A	27-11-1987
		PH 23149 A	11-05-1989
		AT 98495 T	15-01-1994
		AU 592077 B2	04-01-1990
		AU 5566286 A	16-10-1986
		BE 904547 A1	03-10-1986
		CH 672886 A5	15-01-1990
		DE 3689384 D1	27-01-1994
		DE 3689384 T2	07-07-1994
		DK 149886 A	06-10-1986
		EP 0197718 A2	15-10-1986
		EP 0555898 A2	18-08-1993
		ES 8800055 A1	01-01-1988
		FI 861395 A ,B,	06-10-1986

ILL Document Delivery

REG-14343214

CZXKKL

NLM -- W1 IN928M (Gen)

State Technical Library
State Technicial Library
Marianske nam. 5
Prague 1 PSC 110 01
CZECH REPUBLIC

ATTN:
PHONE: 011-42-022-222-1340
FAX: 011-42-022-166-3459
E-MAIL: ill@stk.cz

SUBMITTED: 2008-02-22 14:48:55
PRINTED: 2008-02-25 09:58:56
REQUEST NO.: REG-14343214
SENT VIA: DOCLINE
DOCLINE NO.: KYB-24335734

REG | Copy | Journal

TITLE:	INTERNATIONAL WOUND JOURNAL
PUBLISHER/PLACE:	Blackwell Pub., Oxford :
VOLUME/ISSUE/PAGES:	2007 Jun;4(2):124-37 124-37
DATE:	2007
AUTHOR OF ARTICLE:	Cooper R
TITLE OF ARTICLE:	IODINE REVISITED.
ISSN:	1742-4801
OTHER NUMBERS/LETTERS:	Unique ID.: 101230907 KYB-24335734 17651228
SOURCE:	PubMed
MAX COST:	$14.00
COPYRIGHT COMP.:	Guidelines
CALL NUMBER:	W1 IN928M (Gen)
NOTES:	JE 1-27802210 REF NO: C 985
REQUESTER INFO:	N/A
DELIVERY:	E-mail: ill@stk.cz
REPLY:	Mail:

KEEP THIS RECEIPT TO RECONCILE WITH BILLING STATEMENT
For problems or questions, contact NLM at http://wwwcf.nlm.nih.gov/ill/ill_web_form.cfm or phone 301-496-5511.
Include LIBID and request number.

NLM Collection Access Section, Bethesda, MD

Iodine revisited

Rose A Cooper

Cooper RA. Iodine revisited. Int Wound J 2007;4:124–137.

ABSTRACT

Iodine is an antiseptic that has been used in wound care for more than 150 years. Traditional formulations of iodine had serious limitations that were reduced in later products. Much has been written about iodine and opinions on its clinical efficacy are divided. There have been reviews of the chemical properties of iodine, its antimicrobial activity, human physiology, cytotoxicity and its clinical effectiveness, but few have addressed all these aspects. With the recent development of iodine-containing wound care products and the continued publication of laboratory and clinical studies, it seems timely to reassess the evidence relating to the effectiveness of iodine for treating wounds. This literature review attempts to provide an appropriate chemical and physiological background of the characteristics of iodine in order to provide a sound basis for understanding the available microbiological and clinical data. It will show that understanding the factors that contribute to the activity and potential cytotoxicity of iodine are important in evaluating the clinical evidence. Although definitive studies are needed, the sustained delivery of low doses of free iodine offers the potential to inhibit a broad range of microbial species without selecting for resistant strains or inducing cytotoxic effects.

Key words: Antimicrobial activity • Antiseptic • Cytotoxicity • Iodine • Resistance

Key Points

- Iodine is a chemical element, which occurs naturally as iodide salts in seaweeds, fish, shellfish and seawater
- it is believed that molecular iodine (I_2) has the highest antimicrobial potential
- pH significantly influences the position of dynamic equilibria and therefore also iodine concentration; maximal bactericidal activity occurs when the forms of iodine without bactericidal activity are minimised

THE CHEMISTRY OF IODINE

Iodine is a chemical element, with the chemical symbol I. It occurs naturally as iodide salts in seaweeds, fish, shellfish and also in seawater. Elemental iodine (I_2) was first isolated in 1811. At room temperature it is a dark purple, lustrous, crystalline solid. On heating it melts to form liquid at 113·5°C and boils to a pinkish purple vapour at 184·4°C, but can sublime to vapour directly from the solid, depending on conditions. Its name is derived from the Greek word 'iodes' for violet.

Iodine is the least reactive halogen (other halogens are fluorine, chlorine and bromine). It has an atomic number of 53 and an atomic mass of 126·904. Iodine dissolves readily in ethanol or ether to produce brown solutions, or in chloroform or benzene as violet solutions. It is sparingly soluble in water (0·33 g/l, 1·2 mM, at 25°C) giving a yellowish brown solution (1). Solubility of elemental iodine increases in the presence of iodide ions, such as potassium iodide, where iodine reacts to form tri-iodide ions. Aqueous solutions of iodine are not stable and, depending on conditions, many different species may be present. Of these, it is believed that molecular iodine (I_2) has the highest antimicrobial potential. Stability is influenced by pH and activity diminishes with increased alkalinity and storage time (1). To be able to clearly understand how iodine behaves chemically, its reactions in water have been summarised (Table 1) (2). The seven principal iodine species found in aqueous solution are I_2, HOI, OI^-, H_2OI^+, I_3^-, I^- and IO_3^-, of which only hydrated iodine (I_2), hypoiodous acid (HOI) and iodine cation (H_2OI^+) possess bactericidal activity. At physiologically compatible pH and low concentrations, the only species of importance are I^-, I_2 and I_3^- (3). As H^+ features in four of the above reactions, pH significantly influences the position of dynamic equilibria and therefore also iodine concentration; maximal bactericidal activity occurs when the forms of iodine without bactericidal activity are minimised (2).

Author: RA Cooper, PhD, Department of Applied Sciences, Centre for Biomedical Sciences, Cardiff School of Health Sciences, University of Wales Institute Cardiff, Western Avenue, Cardiff CF5 2YB, UK
Address for correspondence: RA Cooper, Department of Applied Sciences, Centre for Biomedical Sciences, Cardiff School of Health Sciences, University of Wales Institute Cardiff, Western Avenue, Cardiff CF5 2YB, UK
E-mail: rcooper@uwic.ac.uk

THE DEVELOPMENT OF IODINE PRODUCTS FOR WOUND CARE

One of the first antiseptic preparations of iodine was Lugol's solution (1). This tincture of iodine was an aqueous solution of iodine and

Table 1 Potential reactions of molecular iodine (I_2) in water (2)

Category	Reaction	Type of reaction
I	$I_2 + H_2O \leftrightarrow HOI + H^+ + I^-$	Hydrolysis
II	$HOI \leftrightarrow OI^- + H^+$	Dissociation
III	$HOI + H^+ \leftrightarrow H_2OI^+$	Protonation
IV	$I_2 + I^- \leftrightarrow I^-_3$	Complex formation
V	$3HOI \leftrightarrow IO_3^- + 2I^- + 3H^+$	Disproportionation

potassium iodide in ethanol that was used as an antiseptic to treat wounds by Davies in 1839 (4). Subsequently it was used extensively throughout the American Civil War and both Louis Pasteur and Robert Koch are reported to have independently evaluated it (4). It continued to be used professionally and domestically until the 1950s, but it caused acute pain and irritation on application, as well as distinctive staining.

To overcome these clinical limitations, iodophores (or iodine carriers) were developed. Four types of carriers have been used: polyoxymer iodophores, cationic surfactant iodophores, non ionic surfactant iodophores and polyvinyl-pyrrolidone iodophores (otherwise known as polyvinyl-pyrrolidine-iodine, povidone iodine or PVP-I). In most of these agents, iodine is carried in aggregates (or micelles) of detergent which act as reservoirs of iodine. On dilution, these micelles slowly disperse to release free elemental iodine in aqueous solution, so that the concentration of the active agent gradually increases without reaching the undesirable concentrations associated with former products. This free iodine is known as available iodine and the activity of the iodophore is related to the amount of iodine released. Equations to calculate the equilibrium concentration of free iodine released into water from iodine solubilised in surfactants have been derived (5) and titration against sodium thiosulphate with starch indictor allows the concentration of free iodine to be determined experimentally.

In the most common iodophore in clinical use (PVP-I), iodine is chemically bound as triiodide to the surfactant povidone. It was introduced into clinical use in 1956 (6) and it is available as a solution, aerosol spray, ointment, cream or wound dressing. Concentrations vary in different PVP-I preparations, with available iodine ranging between 9·0% and 12% (w/v) (7). It is important to realise that formulations of PVP-I are not identical and that many studies fail to note the precise chemical composition of povidone iodine preparations used. This point was emphasised in a study designed to assess the antibacterial activity of PVP-I where the formulations of PVP-I solution and PVP-I scrub were defined (Table 2), and surfactants were identified (8).

In 1981, cadexomer iodine was developed as another means of delivering 'safe' iodine. It consists of beads of starch containing 0·9% (w/w) iodine. It is available as a powder, ointment and wound dressing. In the wound it readily absorbs fluids to form a gel and as the strands of starch polymer separate on swelling, free iodine in aqueous solution is slowly released (9).

A new generation of iodine products has been developed more recently. In an enzyme-based iodine disinfectant, horseradish peroxidase effects the conversion of iodine from sodium iodide by generating hydrogen peroxide from calcium peroxide (10). An enzyme-mediated system is also used in Oxyzyme™ and Iodozyme™ wound dressings, where glucose oxidase generates hydrogen peroxide using atmospheric oxygen. The hydrogen peroxide, in turn produces oxygen and free iodine (11). An advantage of these products is that inactivated iodide, the reduced form of iodine, can be re-activated by oxidation with further hydrogen peroxide, so that the overall level of iodine/iodide present in the product can be relatively low. In Repithel®, polyvinyl-pyrrolidine-iodine liposomes containing 3% iodine are prepared in a hydrogel (12). Recently a paste comprised of 70% sugar and 30% PVP-I (U-PAST™) has been developed that is claimed to stimulate wound healing by modulating the activity of keratinocytes and fibroblasts (13).

ANTIMICROBIAL ACTIVITY OF IODINE

Iodine has been used extensively as an antiseptic. It has a broad spectrum of antimicrobial activity, rapidly inhibiting bacteria, yeasts, moulds, protozoa and viruses (1,8,14). Enveloped viruses are more susceptible to iodine than non enveloped viruses, probably because of binding of iodine to the lipid component of the envelope. Endospore-forming bacteria generally are less susceptible to antiseptics than non sporing bacteria; however, iodine is an effective sporicidal agent. Inhibition of

Key Points

- one of the first antiseptic preparations of iodine was Lugol's solution; this tincture of iodine was used as an antiseptic to treat wounds
- it caused acute pain and irritation on application, as well as distinctive staining
- to overcome these clinical limitations, iodophores (or iodine carriers) were developed
- In 1981, cadexomer iodine was developed as another means of delivering 'safe' iodine
- a new generation of iodine products have been developed more recently and the advantage of these products is that inactivated iodine, which has been reduced to iodide, can be reactivated by oxidation with further hydrogen peroxide, so that the overall level of iodide present in the product can be relatively low
- a paste comprised of 70% sugar and 30% PVP-I (U-PAST™) has been developed that is claimed to stimulate wound healing by modulating the activity of keratinocytes and fibroblasts

Key Points

- iodine has been used extensively as an antiseptic and has a broad spectrum of antimicrobial activity, rapidly inhibiting bacteria, yeasts, moulds, protozoa and viruses
- iodine is an effective sporicidal agent
- unlike antibiotics where inhibitory effects tend to be localised to a specific cellular location, antiseptics have generalised effects by simultaneously affecting multiple sites in microbial cells
- most of the data demonstrating antimicrobial efficacy have been derived from suspensions of microbial cells tested *in vitro*

Table 2 Typical povidone iodine formulations (8)

Product	Components in the formulation	Concentrations
PVP-I aqueous solution	PVP-I	1% available iodine
	Glycerol	1% (w/v)
	Nonyl phenoxy polyoxyethylene ethanol*	0·25% (v/v)
	Buffer, disodium phosphate/citric acid	Remainder
PVP-I scrub	PVP-I	0·75% available iodine
	Lauric acid diethanolamine condensate*	4% (w/v)
	Ammonium alkyl phenoxy polyoxyethylene glycol sulphonate*	25% (v/v)
	Sodium hydroxide and hydrochloric acid to adjust pH to 4·6	
	Water	Remainder

PVP-I, polyvinyl-pyrrolidine-iodine.
*Surfactants.

mycobacteria has also been reported. Methicillin-resistant staphylococci and methicillin-sensitive staphylococci have been shown to be equally susceptible to iodine (15,16). As little as 0·1 fg (236 000 molecules) of iodine can destroy one bacterial cell (15).

Inhibition of biofilms of *Pseudomonas aeruginosa* and *Burkholderia cepacia* cultivated on Teflon chips has been showed after 10 minutes exposure to PVP-I (0·2%), whereas 60 minutes in contact with chlorhexidine gluconate (0·2%), alkyldiaminoethyleneglycine hydrochloride (0·2%) and benzalkonium chloride (0·2%) had no effect (17). Biofilms have been implicated in chronic wounds and are associated with infections linked to indwelling medical devices (18). They are notoriously difficult to treat because of their reduced susceptibility to antimicrobial agents; iodine seems to offer some potential in limiting biofilms.

MODE OF ACTION OF IODINE

Surprisingly little has been published about its mode of action, but molecular iodine (I_2) is the active agent. At low concentrations, its activity can be affected by organic matter (19). Unlike antibiotics where inhibitory effects tend to be localised to a specific cellular location, antiseptics have generalised effects by simultaneously affecting multiple sites in microbial cells. Binding of iodine to proteins leads to their denaturation in several ways: oxidation of S–H bonds in amino acids such as cysteine and methionine, and the prevention of hydrogen bonding by reacting with N–H groups in arginine, histidine and lysine or the phenolic group of tyrosine. These changes affect the structure and function of both enzymes and structural proteins and therefore have extensive deleterious effects on microbial function. Furthermore, membrane structure is compromised by the reaction of iodine with C=C bonds in fatty acids, and hydrogen bonding in nucleic acids is prevented by iodine binding to nucleotides such as adenine, cytosine and guanine. Hence, changes in cell walls, membranes and cytoplasm result in rapid death following exposure to iodine (1). Structural effects of PVP-I on microbial cells were investigated by electron microscopy and biochemical analysis (20). Rapid partitioning of cytoplasm, coagulation of nuclear material and loss of enzyme activity were found. Cells did not appear to show complete disruption, but pore formation in cell walls led to leakage of selected cellular materials (20).

THE QUALITY OF THE IN VITRO EVIDENCE OF THE ANTIMICROBIAL ACTIVITY OF IODINE

Most of the data demonstrating antimicrobial efficacy have been derived from suspensions of microbial cells tested in vitro. Activity of antimicrobial agents is always influenced by pH, temperature, concentration, contact time, presence of organic matter, electrolytes, microbial strains and the neutralisers used. Experimental conditions, therefore, influence laboratory observations. Despite the publication of numerous studies, critical evaluation of reported data is hampered by incomplete descriptions of methodology and inadequate specification of the formulation of iodine that was used (1).

Disinfectants and antiseptics have been routinely evaluated in the laboratory since the

early 20th century by various methods, but the need to rationalise protocols to provide suitable tests that mimicked the environments in which specific agents were destined to be used has been recognised. In Europe, a range of standardised methods for the laboratory evaluation of antimicrobial agents has gradually been developed since 1995. Initially suspension tests to establish efficacy against bacteria or fungi became available (known as phase 1 tests). In phase 2/step 1 tests, antimicrobial activity in the presence of interfering substances is assayed to determine whether an agent can achieve a 10^5 log reduction of selected test organisms in a given contact time. Phase 2/step 2 tests aim to simulate in vivo conditions before phase 3 tests (clinical trials) are attempted. A complete range of tests is not yet available. Suggested organic challenges for antiseptics that will be used in the oral cavity, on mucous membranes, or on wounds for either prophylactic or therapeutic use have been evaluated with selected antiseptics. A mixture of 4·5% albumin, 4·5% sheep blood and 1% mucin was found to be the most difficult organic challenge and only povidone iodine, octenidine and chlorhexidine retained activity (21). The performance of inhibitory agents in vivo is always less than predicted from laboratory data because in vivo conditions are never faithfully recreated in vitro. Once standardised tests for evaluating antiseptics that are destined to be used on wounds become available, comparisons between agents will become easier. Although the design of laboratory tests can be criticised, there can be little doubt that iodine is a rapid cidal agent.

RESISTANCE TO IODINE

One of the most remarkable features of iodine as an antiseptic is the lack of selection of resistant strains. Only one report of iodine resistance has been published (22). In this report, 10 cultures of methicillin-resistant *Staphylococcus aureus* (MRSA) isolated from different patients were tested against four antiseptics and resistance to PVP-I and sodium hypochlorite together with reduced susceptibility to chlorhexidine acetate and chlorhexidine gluconate was found. However, the experimental conditions used in this study have been criticised because nutrient broth was used which contained components that would have inactivated iodine (15). Another study failed to detect povidone iodine resistance in MRSA (23). Attempts to train bacteria to become resistant to povidone iodine by repeated exposure in the laboratory have failed (24), as have attempts to detect iodine resistance in bacteria isolated from nosocomial infections (25,26). In one study (25), 504 isolates recovered from 12 French hospitals were tested under differing laboratory conditions and inconsistent results were seen. Using a micromethod, 18 strains appeared to be resistant to PVP-I, but none were resistant when a standardised method was adopted (25). The other study was conducted in Italy with 379 isolates recovered from surgical wound infections during a six-year period and no significant variation in susceptibility to antiseptics (including PVP-I) was detected (26). Attempts to show resistance to povidone iodine (0·01%) in coagulase-negative staphylococci isolated from continuous ambulatory peritoneal dialysis patients following long-term prophylactic use of antiseptics at device insertion sites have also failed (27). The consensus is, therefore, that iodine-resistant strains of micro-organisms have not yet emerged. Yet reports of resistance to other antiseptics have been accumulating since the early 1950s (Table 3) and include resistance to quaternary ammonium compounds, chlorhexidine and triclosan in enteric bacteria, pseudomonads and staphylococci (6).

Key Points

- despite the publication of numerous studies, critical evaluation of reported data is hampered by incomplete descriptions of methodology and inadequate specification of the formulation of iodine that was used
- once standardised tests for evaluating antiseptics that are destined to be used on wounds become available, comparisons between agents will become easier
- one of the most remarkable features of iodine as an antiseptic is the lack of selection of resistant strains

THE PHYSIOLOGY OF IODINE

For humans, iodine is an essential trace element that is acquired by eating fish, shellfish and seaweed. The normal daily iodine requirement for adults is between 100 and 200 µg (28). Iodine is absorbed from the blood and concentrated in the thyroid gland where it is used to produce the two thyroid hormones, thyroxine and triiodothyronine, which are important in regulating metabolism. Inadequate iodine intake leads to endemic goiter, endemic cretinism and increased child mortality (29). In developed countries, iodisation of salt (supplementation of sodium chloride with traces of iodide or iodate salts) is an effective strategy in preventing dietary iodine deficiency, because iodised salt is used extensively in processed foods.

Multiple adverse effects are associated with excess iodine including mental depression,

Key Points

- decisions about the choice of a topical antimicrobial intervention by practitioners depend on judgements of safety, effectiveness and appropriateness
- internationally recognised standardised methods, such as ISO 10993, are used for the evaluation of medical devices
- two broad categories of wound care product can be identified: those that contribute to healing and those that contribute to aspects of wound care other than healing

Table 3 Acquired resistance to antiseptics used in wound care

Agent	First clinical use	Resistance	Organisms
Honey	Antiquity	ND	ND
Silver	19th century	1970s	*Pseudomonas aeruginosa* Enterobacteriaceae
Iodine	1839	ND*	ND*
Hydrogen peroxide	1887	ND	ND
Quaternary ammonium compounds	1993	1951	*Ps. aeruginosa*
Chlorhexidine	1954	1967	*Proteus mirabilis*
		1990s	Staphylococci
Triclosan	1970s	1998	*Ps. aeruginosa*

ND, not detected.
*There was a single report of resistance for MRSA in 1985 but of questionable methodology (21).

nervousness, insomnia, myxoedema, hypothyroidism, hyperthyroidism, hypersensitivity and skin reactions (28,30,31). Acute poisoning by ingestion can be fatal (30). Fluctuations in dietary iodine levels, however, are overcome by an autoregulatory mechanism in the thyroid that is known as the Wolff–Chaikoff effect. Although the mechanism of this protective response is not fully understood, it comes into effect when excess iodine levels are ingested. Rather than converting the excess iodine into excess thyroid hormones, the first step in their biosynthetic pathway (oxidation of iodine by organic binding) is temporarily inhibited. Iodine is expelled from the thyroid, removed by the kidneys and excreted in urine. Escape from the Wolff–Chaikoff effect normally ensues after 48 hours, when iodine levels have normalised. Pathological changes arise when autoregulation is defective, for example in the foetus and neonates, and in Hashimoto's thyroiditis, Grave's hyperthyroidism or cystic fibrosis (29). Renal dysfunction in diabetic patients with advanced nephropathy has been linked to non autoimmune primary hypothyroidism (32). Here, elevated serum iodine levels were thought to have resulted from a prolonged Wolff–Chaikoff effect that caused iodine to be expelled from the thyroid, but impaired renal function prevented efficient excretion.

The behaviour of iodine-based antiseptics on skin was investigated by Gottardi in 1995. Using Lugol's iodine solution and PVP-I, uptake (absorption) of free iodine by intact skin was followed by a reversal of the absorption process (or back diffusion). The dynamics of this flux depended on the concentration of free iodine in the preparation applied, contact time and the thickness of the treated area (33).

THE SUITABILITY OF IODINE AS A TOPICAL AGENT IN THE MANAGEMENT OF WOUNDS

Decisions about the choice of a topical antimicrobial intervention by practitioners depend on judgements of safety, effectiveness and appropriateness. The way in which those attributes are assessed depends to a certain extent on the methods used. It should be remembered that proprietary wound care products satisfy criteria set by regulatory bodies before being licensed. Often, however, these supporting data never reach the public domain and comparisons between products by potential users have to be made from evidence generated by clinical researchers. Historically evaluations of safety and clinical efficacy used a variety of diverse tests. Now internationally recognised standardised methods, such as ISO 10993, are used for the evaluation of medical devices. ISO 10993 is comprised of 18 parts: parts 1–12 concern biological testing and 13–18 chemical characterisations. Similarly European legislation was laid down to ensure the safety of medical devices (90/385/EEC and 93/42/EEC) and essentially the tests have been harmonised with ISO 10933. Two broad categories of wound care product can be identified: those that contribute to healing and those that contribute to aspects of wound care other than healing. The biological evaluation of medical devices includes tests for genotoxicity, carcinogenicity and reproductive toxicity (ISO 10993 part 4), in vitro cytotoxicty (part 5), irritation and sensitisation (part 10) and systemic toxicity

(part 11). A comprehensive evaluation strategy for a topical antimicrobial solution determines antimitotic activity of the agent using monolayer cell cultures of each of the cell types that occur in the target tissue (human or mouse fibroblasts, keratinocytes and polymorphonuclear leukocytes). The effect of an agent on processes pertinent to wound healing is tested (i.e. cell migration, angiogenesis, synthesis of extracellular matrix components and wound closure) using three-dimensional models. Finally, in vivo studies on animal models are performed and clinical evidence was collected from humans.

SAFETY OF IODINE: EVIDENCE FROM IN VITRO CYTOTOXICITY STUDIES

Although antiseptics in general have a reputation as cytotoxic agents, the evidence to support that assumption for iodine is not overwhelming because both negative and positive reports have been published. By exposing granulocytes and monocytes to a range of concentration of three preparations of PVP-I, it was demonstrated that the concentrations used clinically (0·1–20% v/v) were toxic. In the presence of lower concentrations (0·005% v/v), viability and phagocytic activity were retained after 60 minutes, as well as antibacterial activity (34). This study indicated that toxicity was related to PVP-I concentration and suggested that dilute solutions may be clinically effective. However, growth of human adult skin fibroblasts and foetal lung fibroblasts was progressively retarded by 0·01% and 0·025% PVP-I and completely inhibited at higher concentrations, which suggested that even dilute solutions of PVP-I were toxic (35). Cytotoxicity of 1% PVP-I towards human fibroblasts obtained from newborn foreskins has also been published (36); similarly, negative effects of diluted PVP-I solutions (and a range of other topical antimicrobial agents) against human fibroblasts and keratinocytes were reported (37). A toxicity index for 20 skin and wound cleansers has been derived using human infant fibroblasts and keratinocytes (38). Using the viability of cells exposed to saline as a baseline (no toxicity), the viability of cells exposed to a series of dilutions of cleansers was determined. The dilution factor of test solution that did not affect viability was deduced to be the toxicity index. Hence, highly toxic agents would require high dilution (high toxicity index) before inhibition (viability) of cells was prevented, but non toxic agents (low toxicity index) required less dilution. In this study, 10% povidone, PVP-I surgical scrub and hydrogen peroxide (3%) were all 100 times less toxic than household bath soaps but 1000 times more toxic than saline (38).

Most investigations into cytotoxicity have used cells directly involved in wound repair, but the effect of topical antimicrobial agents on human neutrophils isolated from blood was tested in an attempt to discover whether host defence cell function was affected. PVP-I solutions at or below clinically relevant concentrations did inhibit respiratory burst (39). Any loss of this important function would impact on the killing of ingested micro-organisms within these cells, and so was considered to undermine antiseptic potential.

Not all reports confirm cytotoxic effects of iodine in vitro. Exposing human fibroblasts to a range of concentrations of cadexomer iodine in vitro showed that viability and collagen synthesis were unaffected at 0·45%, which was thought to illustrate its lack of toxicity in vivo (40). Two studies have suggested potentially beneficial effects of iodine on wound healing. The human macrophage cell line U937 exposed to 0·25% cadexomer iodine (Iodosorb) was stimulated to secrete proinflammatory cytokines (tumour necrosis factor-alpha or TNF-α) in response to 0·00225% iodine (41). Macrophages stained in biopsies taken from chronic wounds are negative for TNF-α, therefore a role for iodine in stimulating the activation of macrophages in non healing wounds was postulated by these researchers. Cadexomer iodine formulations might also promote healing by modulating the redox environment. Investigation into the pro- and antioxidant activities of cadexomer iodine, its constituents and of excipients present in commercial preparations showed some interesting effects on L929 mouse fibroblasts and mouse macrophages (42). The modified starch in cadexomer iodine was found by two assays to lack free radical scavenging antioxidant activity and did not generate hydrogen peroxide through autooxidation. Cadexomer iodine (0·05–2% w/v) enhanced proliferation in the fibroblasts, while Iodosorb powder (1–2% w/v) and iodine alone (0·009–0·018% w/v) inhibited superoxide generation in stimulated macrophages. Iodine was

Key Points

- although antiseptics in general have a reputation as cytotoxic agents, the evidence to support that assumption for iodine is not overwhelming because both negative and positive reports have been published

Key Points

- in relation to wounds, comments on the safety of iodine seem largely to concern povidone iodine not cadexomer iodine, but once again conflicting evidence exists

postulated to cause these effects by the oxidation of intracellular reducing agents, such as reduced nicotinamide adenine dinucleotide phosphate (NAD(P)H) and glutathione. A further antioxidant effect (singlet oxygen scavenging) was attributed to excipients (42). Although not made in a chronic wound, these observations indicate that iodine can influence the formation of free radicals derived from oxygen, and so modulate the function of cells involved in healing. An inference is that oxygen influences the function of iodine in vivo.

It can be argued that cells in culture do not behave as cells in vivo, because their susceptibility is reduced in the absence of homeostatic mechanisms. Fibroblasts, for example, are protected by the upper layers of the skin in vivo. To overcome some of these criticisms, three-dimensional collagen lattices seeded with human fibroblasts were developed as wound-healing models (43). The immortalized mouse fibroblast cell line L929 has been used extensively for cytotoxicity testing. Yet immortalized cells and cell lines derived from human explants may not accurately represent a chronic wound. Hence, a fibroblast gel contraction model using equine fibroblasts collected from granulation tissue in a slow-healing wound was used to test several topical iodine antiseptics. The results indicated that prolonged treatment with iodine might be detrimental to wound healing (44).

SAFETY OF IODINE: EVIDENCE FROM IN VIVO ANIMAL MODELS

Irrigation of guinea pig wounds with iodine antiseptics was found to be effective in preventing wound infection (45). Of four antiseptics applied to clean wounds created in white domestic pigs, PVP-I was not found to affect the rate of healing (46). The clinical use of antiseptics was profoundly influenced by two studies that were published in 1985 in which animal models were used to evaluate the toxicity of antiseptics (36,47). Using incisions on the backs of rats, irrigation with solutions of 1% PVP-I, 0·25% acetic acid, 0·5% sodium hypochlorite or 3% hydrogen peroxide showed retarded epithelialisation and reduced wound strength (36). A rabbit ear chamber (47) allowed the effect of antiseptics (EUSOL, PVP-I 1% and 5%, hydrogen peroxide 10 vol, Chloramine T 1% and chlorhexidine 0·05%) to be determined on granulation tissue. Direct microscopic observation showed that blood flow was markedly affected (particularly by EUSOL and Chloramine T, where empty capillaries did not recover in 10 days). In these studies, all the antiseptics tested showed adverse effects in comparison to saline; recommendations to restrict the use of antiseptics were made. Some practitioners interpreted these findings as a warning not to use antiseptics in wounds (28). The anionic detergent present in PVP-I surgical scrub has been implicated in increased inflammation in guinea pigs (48). Another indication that a specific surfactant might contribute to toxicity came from cytotoxic effects of three PVP-I formulations on guinea pig wounds. Polyoxyethylene nonylphenyl ether was reported to be 100 times more toxic than sodium polyoxyethylene lauryl ether sulphate (49).

Efficacy of cadexomer iodine was showed on partial thickness wounds in pigs challenged with MRSA (50). Positive effects of cadexomer iodine on epidermal regeneration during healing in full thickness, non infected wounds in the pig have also been reported (51).

SAFETY OF IODINE: EVIDENCE OF ADVERSE EFFECTS FROM TREATED PATIENTS

In relation to wounds, comments on the safety of iodine seem largely to concern povidone iodine not cadexomer iodine, but once again conflicting evidence exists. Reports of systemic effects following short-term use of PVP-I are rare. Fatalities have been attributed to topical use of PVP-I in two burns patients (52) and following surgical debridement of a hip wound (53). Mediastral irrigation with PVP-I has been reported to result in acute renal failure (54) and seizures (55). Elevated serum iodine has been linked to renal impairment and hyperchloremic acidosis following the use of PVP-I (56,57), and it has been suggested that long-term topical treatment with PVP-I on 40 neurological in-patients caused mild thyroid dysfunction (58). Investigations into the extent of iodine absorption through wounds do not yield conclusive evidence of adverse systemic effects. Iodine levels were monitored in the blood and urine of 33 burns patients and undesirable thyroid or renal effects were not detected (59). Likewise, changes in the levels of

thyroid hormones of 10 patients with extensive third-degree burns that were treated with PVP-I were not found (60), and the use of PVP-I in 18 paediatric cardiac patients did not lead to altered thyroid function (61). Serum and urine iodine levels after topical application of PVP-I were deduced to be related to the size of a burn and renal function, but effects on thyroid function were not found (62). Increased levels of serum iodide in burns patients relate not only to the size of the affected area but also to the length of treatment (63). Although serum iodide levels can be expected to return to normal following cessation of treatment with PVP-I, patients with existing thyroid disease, pregnant women, nursing mothers and infants were considered unsuitable candidates for long-term topical application of povidone-iodine (63). Adverse effects noted in case reports may have been associated with underlying pathologies, rather than iodine alone because some patients had multiple aetiologies (63). Recommendations that iodophores should be used in neither patients with renal damage nor those with extensive burns (52,62) are sensible.

Allergic reactions to iodine have also been reported, with prevalence reports ranging from 0·7% to 41% (63). A high prevalence of sensitisation to topical agents in leg ulcer patients prompted a French group to analyse published studies and to review their own patients (64). Patch testing in three groups of patients with the European series of standards and an additional series of potential allergens pertinent to leg ulcers showed that Balsam of Peru, fragrance mix and nickel sulphate had sensitisation rates above 10%, whereas PVP-I as Betadine® had lower rates than neomycin or Cetavlon®, but not chlorhexidine digluconate or Flamazine® (64). In Hungary, the successful use of Betadine® with dermatology patients over many years was reported (65); to determine whether any patients had been sensitised to PVP-I, 50 were challenged by patch testing and no sensitisation was found.

Doubts about the validity of positive patch tests where PVP-I (10% solutions in petrolatum, i.e. 1% free iodine) are tested under occlusion caused Lachapelle to test 500 consecutive patients with conventional patch tests (66). Only 14 positive patients were found; each of them was retested in a repeated open application test where PVP-I dermal solution was applied to the open forearm twice daily for 7 days. Two of these tests were positive, thus a prevalence rate of 0·4% with true allergic contact dermatitis to PVP-I was deduced. It has been suggested that PVP-I containing detergents caused cytotoxicity and sensitisation in wounds but not intact skin (67). Testing panels must always include components contributing to the manufacture of modern dressings. Fears that manufacturers fail to declare all ingredients in their formulations may confound sensitivity testing (68). It must also be remembered that sensitisation may occur before treatment regimes commence and that allergen tests reflect not only health care experiences. Reports of iodine allergy may, therefore, be exaggerated.

EVIDENCE OF THE EFFICACY OF IODINE FROM CLINICAL STUDIES

The role of iodine in wound care is predominantly an antimicrobial agent. Solutions, sprays and scrubs have been used to irrigate contaminated trauma wounds, and prophylactically to reduce skin flora immediately before and sometimes after surgery. Iodine-containing ointments, creams and dressings are intended to prevent ingress of pathogens into wounds, to act as a barrier to cross-infection, and to prevent the progression from localized to overt infection. Infection interrupts healing and extends the time to wound closure therefore preventing infection prevents the extension of the healing period. A correlation between decrease in bacterial load and the rate of wound healing was established with the topical application of furazolidone to 56 pressure sores and stasis ulcers in 47 alcoholic or neuropsychiatric patients (69). This principle provides the rationale for the use of topical antimicrobial agents in wounds, yet few studies monitor the quantitative effects of topical agents on microbial flora. The efficacy of five antiseptic solutions and four antimicrobial creams in eradicating coagulase-negative staphylococci from the stratum corneum was investigated in one study, though (70). Four of the agents contained iodine (solutions of 10% PVP-I, 2% aqueous iodine, 2% tincture of iodine and iodophore ointment were used). All nine agents successfully eradicated the bacteria from surface layers, but only 2% iodine, mupirocin and a triple antibiotic ointment removed bacteria

Key Points

- adverse effects noted in case reports may have been associated with underlying pathologies, rather than iodine alone because some patients had multiple aetiologies
- fears that manufacturers fail to declare all ingredients in their formulations may confound sensitivity testing
- reports of iodine allergy may, therefore, be exaggerated
- the role of iodine in wound care is predominantly an antimicrobial agent
- iodine-containing ointments, creams and dressings are intended to prevent ingress of pathogens into wounds, to act as a barrier to cross-infection, and to prevent the progression from localized to overt infection

Key Points

- cadexomer iodine has been shown to enhance healing rates in chronic wounds, particularly venous leg ulcers; it has been shown to be an efficient, cost-effective and safe alternative to hydrocolloid dressing and paraffin gauze dressing for the treatment of chronic leg ulcers

from within the layers of the skin. Repopulation by resident flora occurred within 24 hours following use of 2% iodine and iodophore; PVP-I and tincture were not tested (70). Hence, the temporary nature of antimicrobial effects was illustrated.

Much of the data on the effectiveness of iodine have been generated from randomised controlled trials (RCTs) in surgical patients, and therefore relates to acute wounds. It is neither conclusive nor consistent. Preoperative antibiotics were found to be superior to PVP-I in preventing postoperative wound infections following abdominal surgery (71). Considering the high levels of contamination possible in this situation, it is plausible that systemic rather than localized antimicrobial intervention is required. However, in appendectomies bacterial contamination depends on the inflammation associated with the appendix and can be mild or slight. Viljanto (72) investigated the efficacy of PVP-I in paediatric patients with appendicitis who did not have either peritonitis or periappendicular abscesses. In these moderately contaminated wounds, a 5% PVP-I solution containing excipients (glycerol, citrate phosphate buffer, polyoxyethylated nonylphenol) affected healing more than 1% PVP-I without excipients (72). Intraperitoneal irrigation with 0·1% PVP-I solution was compared with saline in a prospective randomised clinical trial with 168 consecutive laparotomy patients and significantly fewer intra-abdominal abscesses were seen with PVP-I. Increased serum iodine levels were noted 24 hours after irrigation, but normal levels were regained within 72 hours and no changes in thyroxine were seen (73). Povidone iodine was found to be a safe and effective way to prevent postoperative wound infection following gastrointestinal surgery (74), but less convincing data were generated in another study (75).

The benefits of PVP-I in the treatment of traumatic wounds have been investigated. In a prospective RCT of 500 consecutive patients attending an emergency department with lacerations requiring sutures, a 60-second irrigation with 1% PVP-I and scrubbing gave rise to less wound infection than saline without scrubbing (76). The effect of soaking traumatic wounds in either 1% PVP-I or saline, or no soaking showed no statistically significant difference between numbers of bacteria in PVP-I-treated wounds and controls (77).

Healing rates in patients following toenail surgery by matrix phenolisation were compared in an RCT using either medicated honey dressings or PVP-I impregnated dressings. Statistically significantly accelerated healing rates were found with iodine compared with honey in patients with total nail avulsion, but not in partial avulsion (78).

Disinfection of skin at incision sites before surgery has a long history, but a review of the effectiveness of preoperative antiseptics in preventing postoperative wound infections after clean surgery concluded that there was insufficient data to draw firm conclusions (79).

Fewer clinical studies on the efficacy of iodine in chronic wounds have been performed. Quantitative bacteriology was used in a prospective RCT of the topical treatment of pressure sores with 40 patients. Silver sulphadiazine was more effective in reducing bacterial load than PVP-I or saline, but the numbers of patients were low (80). Burns and chronic ulcers treated over 5 years with granulated sugar and PVP-I or PVP-I alone had reduced the need for grafting and antibiotics (81). Cadexomer iodine has been shown to enhance healing rates in chronic wounds, particularly venous leg ulcers (82–84). It has been shown to be an efficient, cost-effective and safe alternative to hydrocolloid dressing and paraffin gauze dressing for the treatment of chronic leg ulcers (84).

The continued debate on the deleterious effects elicited by both micro-organisms and antiseptics on human cells intimately involved in the healing process motivated an investigation into the efficacy of iodine using histological and inflammatory markers. In 15 female patients with at least two chronic ulcers, either hydrocolloid dressing alone or hydrocolloid dressing with daily application of PVP-I solution were used in each of the two wounds and biopsies were collected after 4 weeks of treatment. Size of ulcer reduced faster in wounds with hydrocolloid and PVP-I, together with the observation of fewer bacteria and less pronounced inflammatory effects. Reduction in bacteria-related inflammation was thought to promote the enhanced healing rate (85). In a further study by this group, the effect on healing in chronic leg ulcers of three antiseptics (PVP-I, silver sulfadiazine and chlorhexidine digluconate) was investigated. Compared with controls, PVP-I significantly increased healing

rate and reduced time to healing. All the antiseptics caused decreased bacterial density in biopsies collected from the treated wounds with concordant-abated vasculitic changes. Only PVP-I, however, did not significantly reduce the density of dendrocytes and fibroblasts, therefore selective, moderate cytotoxicity in vivo was argued to result in a paradoxically beneficial outcome (86).

A novel property attributed to povidone-iodine that supports its use in chronic non healing wounds is its ability to reduce protease activity. Depending on concentration, PVP-I has been shown by zymography to inhibit metalloprotease activity in samples of wound fluid obtained from non healing wounds and to reduce neutrophil elastase and plasmin activity (87).

Although antimicrobial interventions have long been used on chronic wounds, a systematic review of antimicrobial agents commonly used in the management of chronic wounds concluded that there were insufficient clinical data to recommend any agent (88).

CLINICAL USE OF IODINE TODAY

Advice on using iodine-based wound care products can be found in the British National Formulary. Newer formulations of iodine have not yet become widely integrated into local formularies and clinical data are limited to date. Povidone iodine is mainly used in skin disinfection and in acute wounds, such as contaminated traumatic wounds or surgical incisions; cadexomer iodine is more commonly used in chronic wounds. Both are used prophylactically, and can be used to limit localized infection. Another reason for using iodine might be in non healing wounds where other limiting influences have been addressed, because underlying subclinical infection might impede healing. Neither PVP-I nor cadexomer iodine should be used repeatedly over long periods in wounds that remain unchanged. Both offer benefits in terms of cleansing and dedridement and can be used with compression therapy and pressure relief (89). Cadexomer iodine dressings have considerable absorptive characteristics (82), but highly exudating wounds will elute PVP-I from impregnated dressings (90). An evaluation of clinical evidence indicated that many antiseptics including cadexomer iodine and PVP-I do not impede healing, and that cadexomer iodine promotes healing (91). Where a specific wound product has been showed to offer no clinical advantage over comparable products, relative costs become important and cadexomer iodine has been shown to be cost-effective in relation to other topical agents (92).

EVALUATING INFORMATION ABOUT THE EFFICACY OF IODINE

In caring for wounds, practitioners have to consider many factors in deriving an effective therapeutic strategy that provides optimum conditions to support rapid healing (93). Undoubtedly systemic antibiotics are indicated in cases of overt wound infection (94), and all antimicrobial agents must be used sparingly to avoid the selection of resistant strains. Whereas iodine used to be considered to be inappropriate for wound care (95,96), attitudes are changing as a result of the analysis of accumulated data.

The effects of PVP-I on healing have been reviewed (97). Solution, scrubs, ointment and cream were considered in animal and human studies and it was concluded that PVP-I did not have a deleterious effect on wound healing (97). A report of a consensus meeting on the use of iodine in wound care that was organized by the European Tissue Repair Society was largely supportive of iodine (98). It was deduced that slow-release formulations that generate low concentrations of iodine in a wound were effective and non toxic.

Reviews of animal and clinical data indicate that despite reported cytotoxicity, common antiseptics (particularly those containing iodine) do not appear to impede healing (90,91,99–101), especially when used appropriately (99). Although PVP-I has been judged to be relatively safe for small acute wounds, caution is evident in relation to use in more extensive and chronic wounds (100). Iodine has been described as one of the most powerful antiseptics available (28). A review of 22 clinical studies using PVP-I and 13 studies using cadexomer iodine evaluated effects on wound healing and re-epithelialisation, as well as efficacy in reducing bacterial loads and the incidence of infection (91). For PVP-I-negative effects on wound healing were not found, and cadexomer iodine accclerated healing. The authors concluded that 'In the majority of clinical trials, antiseptics appear to be safe and

Key Points

- newer formulations of iodine have not yet become widely integrated into local formularies and clinical data are limited to date
- neither PVP-I nor cadexomer iodine should be used repeatedly over long periods in wounds that remain unchanged
- an evaluation of clinical evidence indicated that many antiseptics including cadexomer iodine and PVP-I do not impede healing, and that cadexomer iodine promotes healing
- cadexomer iodine has been shown to be cost-effective in relation to other topical agents
- iodine used to be considered inappropriate for wound care but attitudes are changing as a result of the analysis of accumulated data
- a report of a consensus meeting on the use of iodine in wound care that was organized by the European Tissue Repair Society was largely supportive of iodine
- it showed that slow-release formulations that generate low concentrations of iodine in a wound were effective and non toxic

Key Points

- the authors concluded that 'In the majority of clinical trials, antiseptics appear to be safe and were not found to negatively influence wound healing'
- increased understanding of the factors that influence the activity of iodine (e.g. pH, oxygen, free iodine concentration) together with those that contribute to cytotoxicity (e.g. presence of surfactants) will allow the development of better products
- formulations that deliver a low, sustained dose of iodine have the potential to provide effective antimicrobial activity without significant cytotoxicity
- perhaps its greatest strength, after over 150 years of use in humans, is that there is no evidence that bacteria have found a way of developing resistance

were not found to negatively influence wound healing' (91).

An evaluation of the evidence for PVP-I contained in 41 studies together with another 14 literature sources used for background reading has recently been published (101). A hierarchy of experimental studies, descriptive studies and expert evidence was derived using defined criteria in order to judge the quality of evidence. Overall 49% of articles did not support the use of PVP-I, whereas 71% of the 'better' quality articles did support PVP-I (101).

THE FUTURE FOR IODINE

A recurring theme in the analysis of the evidence for any wound care treatment seems to be the paucity of good quality data, the poor design of studies and the low numbers of patients treated. Yet many dressings have gained acceptance without objective evidence (102,103). Definitive clinical studies (RCTs) are essential to substantiate claims of efficacy of iodine. However, increased understanding of the factors that influence the activity of iodine (e.g. pH, oxygen, free iodine concentration) together with those that contribute to cytotoxicity (e.g. presence of surfactants) will allow the development of better products. Formulations that deliver a low, sustained dose of iodine have the potential to provide effective antimicrobial activity without significant cytotoxicity.

CONCLUSION

Iodine should no longer be regarded as an old-fashioned antiseptic. Formulations of iodine in earlier wound care products had serious limitations, but newer formulations have reduced those disadvantages. By the sustained delivery of free iodine at concentrations that retain antimicrobial activity without cytotoxicity for mammalian cells, it is possible to reduce microbial load and to modulate host cells to elicit responses that stimulate healing. When used appropriately, iodine seems to offer potential as an effective, broad-spectrum antimicrobial agent that can promote healing. Perhaps its greatest strength, after over 150 years of use in humans, is that there is no evidence that bacteria have found a way of developing resistance.

ACKNOWLEDGEMENTS

This study was sponsored by Insense Ltd.

REFERENCES

1 Gottardi W. Chapter 8: iodine and iodine compounds. In: Block SS, editor. Disinfection, sterilization and preservation, 3rd edn. Philadelphia: Lea & Febiger, 1983:183–96.
2 Gottardi W. The influence of the chemical behaviour of iodine on the germicidal action of disinfectant solutions containing iodine. J Hosp Infect 1985;6(Suppl):1–11.
3 Gottardi W. Iodine and disinfection: theoretical study on mode of action, efficiency, stability, and analytical aspects in the aqueous system. Arch Pharm 1999;332:151–7.
4 Hugo WB. A brief history of heat and chemical preservation and disinfection. J Appl Bacteriol 1991;71:9–18.
5 Allawala NA, Riegelman S. The properties of iodine in solutions of surface active agents. J Am Pharm Assoc 1953;42:396–401.
6 Russell AD. Introduction of biocides into clinical practice and the impact on antibiotic-resistant bacteria. J Appl Bacteriol 2002;92(Symp Suppl): 121S–135S.
7 British Pharmacopoeia. Povidone iodine. p1636 volume II. London: The Stationery Office, 2005.
8 Saggers BA, Stewart GT. Polyvinyl-pyrrolidone-iodine: an assessment of antibacterial activity. J Hyg 1964;62:509–19.
9 Mårtenson L. Cadexomer iodine – an introduction. In: Iodine and wound physiology. Cambridge: Information transfer Ltd, 1995:1.1–1.2.
10 Duan Y, Dinehart K, Hickey J, Panicucci R, Kessler J, Gottardi W. Properties of an enzyme-based low-level iodine disinfectant. J Hosp Infect 1999; 43:219–29.
11 Thorn RMS, Greenman J, Austin A. An in vitro study of antimicrobial activity and efficacy of iodine generating hydrogel dressings. J Wound Care 2006;15:305–10.
12 Vogt PM, Reimer K, Hauser J, Rosenbach O, Steinau HU, Bosse B, Muller S, Schmidt T, Fleischer W. PVP-iodine in hydrosomes and hydrogel-A novel concept in wound therapy leads to enhanced epithelialisation and reduced loss of skin grafts. Burns 2006;32:698–705.
13 Nakao H, Yamazaki M, Tsuboi R, Ogawa, H. Mixture of sugar and povidone-iodine stimulates wound healing by activating keratinocytes and fibroblast functions. Arch Dermatol Res 2006; 298:175–82.
14 Zamora JL. Chemical and microbiologic characteristics and toxicity of povidone-iodine solutions. Am J Surg 1986;151:400–6.
15 Lacey RW, Cato A. Action of povidone-iodine against methicillin-sensitive and resistant cultures of *Staphylococcus aureus*. Postgrad Med J 1993; 69(Suppl):S78–83.
16 Yasuda T, Yoshimura S, Katsuno Y, Ito M, Takada H, Takahashi M, Yahazaki F, Iriyama J, Ishigo S, Asano Y. Comparison of bactericidal activities of various disinfectants against methicillin-resistant and methicillin-sensitive *Staphylococcus aureus*. Postgrad Med J 1993;69(Suppl): S66–9.

17 Kunisada T, Yamada K, Oda S, Hara O. Investigation into the efficacy of povidone-iodine against antiseptic-resistant species. Dermatology 1997; 195(Suppl):14–8.
18 Okhiria O, Cooper R. Biofilms, wound infection and the issue of control. Wounds 2006;2:48–57.
19 Moore SL, Payne DN. Chapter 2: types of antimicrobial agents. In: Fraise AP, Lambert PA, Maillard J-Y, editors. Russell, Hugo & Ayliffe's principles and practice of disinfection, preservation & sterilization, 4th edn. Oxford: Blackwell Publishing, 2004:8–97.
20 Schreier H, Erdos G, Reimer K, Konig B, Konig W, Fleischer W. Molecular effects of povidone-iodine on relevant micro-organisms: an electron-microscopic and biochemical study. Dermatology 1997;195(Suppl):111–6.
21 Pitten F-A, Werner H-P, Kramer A. A standardized test to assess the impact of different organic challenges on the antimicrobial activity of antiseptics. J Hosp Infect 2003;55:108–15.
22 Mycock G. Methicillin/antiseptic-resistant *Staphylococcus aureus*. Lancet 1985;2:949–50.
23 McLure AR, Gordon J. *In-vitro* evaluation of povidone-iodine and chlorhexidine against methicillin-resistant *Staphylococcus aureus*. J Hosp Infect 1992;21:291–9.
24 Houang E, Gilmore OJA, Reid C, Shaw EJ. Absence of bacterial resistance to povidone iodine. J Clin Pathol 1976;29:752–5.
25 Traoré O, Fayard SF, Laveran H. An in-vitro evaluation of the activity of povidone-iodine against nosocomial bacterial strains. J Hosp Infect 1996;34:217–22.
26 Giacometti A, Cirioni O, Greganti G, Fineo A, Ghiselli R, Del Prete MS, Mocchegiani F, Fileni B, Caselli F, Petrelli E, Saba V, Scalise G. Antiseptic compounds still active against bacterial strains isolated from surgical wound infections despite increasing antibiotic resistance. Eur J Clin Microbiol Infect Dis 2002;21:553–6.
27 Klossner BL, Widmer H-R, Frey F. Nondevelopment of resistance by bacteria during hospital use of povidone-iodine. Dermatology 1997;195(Suppl): 10–3.
28 Lawrence JC. The use of iodine as an antiseptic agent. J Wound Care 1998;7:421–5.
29 Woeber KA. Iodine and thyroid disease. Med Clin North Am 1991;75:169–78.
30 Richardson ML, Gangolli, S. Iodine. In: Richardson ML, Gangolli S, editors. The dictionary of substances and their effects. Vol 5. Cambridge: The Royal Society of Chemistry, 1994:47.
31 Weeke J. Chapter 41: thyroid and anti-thyroid drugs. In: Dukes MNG, Aronson JK, editors. Meyler's side effects of drugs, 14th edn. Amsterdam: Elsevier Science BV, 2000;1492–1493.
32 Bando Y, Ushiogi Y, Okafuji K, Toya D, Tanaka N, Miura S. Non-autoimmune primary hypothyroidism in diabetic and non-diabetic chronic renal dysfunction. Exp Clin Endocrinol Diabetes 2002; 110:408–15.
33 Gottardi W. The uptake and release of molecular iodine by the skin: chemical and bactericidal evidence of residual effects caused by povidone-iodine preparations. J Hosp Infect 1995;29:9–18.
34 Van den Broek PJ, Buys LFM, Van Furth R. Interaction of povidone-iodine compounds, phagocytic cells, and micro-organisms. Antimicrob Agents Chemother 1982;22:593–7.
35 Balin AK, Pratt L. Dilute povidone-iodine solutions inhibit human skin fibroblast growth. Dermatol Surg 2002;28:210–4.
36 Lineaweaver W, Howard R, Soucy D, McMorris S, Freeman J, Crain C, Robertson J, Rumley T. Topical antimicrobial toxicity. Arch Surg 1985; 120:267–70.
37 Cooper ML, Laxer JA, Hansborough JF. The cytotoxic effects of commonly used topical antimicrobial agents on human fibroblasts and keratinocytes. J Trauma 1991;31:775–84.
38 Wilson JR, Mills JG, Prather ID, Dimitrijevich SD. A toxicity index of skin and wound cleansers used on in vitro fibroblasts and keratinocytes. Adv Skin Wound Care 2005;18:373–8.
39 Hansborough JF, Zapata-Sirvent RI, Cooper ML. Effects of topical antimicrobial agents on human neutrophil respiratory burst. Arch Surg 1991;126: 603–8.
40 Zhou LH, Nahm WK, Badiavas E, Yufit T, Falanga V. Slow release iodine preparation and wound healing: *in vitro* effects with lack of *in vitro* toxicity in human chronic wounds. Br J Dermatol 2002; 146:365–74.
41 Moore K, Thomas A, Harding KG. Iodine released from the wound dressing Iodosorb modulates the secretion of cytokines by human macrophages responding to bacterial lipopolysaccharide. Int J Biochem Cell Biol 1997;29:163–71.
42 Schmidt RJ, Kirby AJ, Chung LY. Cadexomer iodine formulations may modulate the redox environment of wounds. In: Iodine and wound physiology. Cambridge: Information transfer Ltd, 1995: 6.1–6.26.
43 Bell E, Ivarsson B, Merrill C. Production of a tissue-like structure by contraction of collagen lattices by human fibroblasts of different proliferative potential in vitro. Proc Natl Acad Sci USA 1979;76: 1274–8.
44 Cochrane CA, Shearwood C, Walker M, Bowler P, Knottenbelt DC. The application of a fibroblast gel contraction model to assess the cytotoxicity of topical antimicrobial agents. Wounds 2003;15: 265–71.
45 Edlich RF, Custer J, Madden J, Dajani AS, Rogers W, Wangensteen OH. Studies in management of the contaminated wound. Am J Surg 1969;118: 21–30.
46 Geronemus RG, Mertz PM, Eaglstein, WH. Wound healing. The effects of topical antimicrobial agents. Arch Dermatol 1979;115:1311–4.
47 Brennan SS, Leaper DJ. The effect of antiseptics on the healing wound: a study using the rabbit ear chamber. Br J Surg 1985;72:780–2.
48 Custer J, Edlich RF, Prusak M, Madden J, Panek P, Wangensteen OH. Studies in the management of the contaminated wound. Am J Surg 1971;121: 572–5.

49 Iwasawa A, Nakamura Y. Cytotoxic effect and influence of povidone-iodine on wounds in guinea pig. J Jpn Assoc Infect Dis 2003;77:948–56.

50 Mertz PM, Oliveira-Gandia MF, Davis SC. The evaluation of a cadexomer iodine wound dressing on methicillin resistant Staphylococcus aureus (MRSA) in acute wounds. Dermatol Surg 1999;25: 89–93.

51 Lamme EN, Gustafsson TO, Middelkoop E. Cadexomer-iodine ointment shows stimulation of epidermal regeneration in experimental full-thickness wounds. Arch Dermatol Res 1998;290:18–24.

52 Pietsch J, Meakins JL. Complications of povidone-iodine absorption in topically treated burns patients. Lancet 1976;1:280–2.

53 D'Auria J, Lipson S, Garfield JM. Fatal iodine toxicity following debridement of a hip wound: case report. J Trauma 1990;30:353–5.

54 Campistol JM, Abad C, Nogue S, Bertran A. Acute renal failure in a patient treated by continuous povidone-iodine mediastral irrigation. J Cardiovasc Surg (Torino) 1988;28:410–2.

55 Zec N, Donovan JW, Aufiero TX, Kincaid RL, Demers LM. Seizures in a patient treated with continuous povidone-iodine mediastral irrigation. N Engl J Med 1992;326:1784.

56 Lavelle KJ, Doedens DJ, Kleit SA, Forney RB. Iodine absorption in burns patients treated topically with povidone iodine. Clin Pharmacol Ther 1975;17: 355–62.

57 Aronoff GR, Freidman SJ, Doedens DJ, Lavelle KJ. Increased serum iodide concentration from iodine absorption through wounds treated topically with povidone-iodine. Am J Med Sci 1980;279:173–6.

58 Nobukuni K, Hayakawa N, Namba R, Ihara Y, Sato K, Takada H, Hayabara T, Kawahara S. The influence of long-term treatment with povidone-iodine on thyroid function. Dermatology 1997; 195(Suppl):60–72.

59 Zellner PR, Bugyi S. Povidone-iodine in the treatment of burns patients. J Hosp Infect 1985;6 (Suppl):139–46.

60 Balogh D, Bauer M, Riccabona G. The influence of povidone-iodine treatment on thyroid hormones in severe burns. J Hosp Infect 1985;6(Suppl):147–53.

61 Kovacikova L, Kunovsky P, Skrak P, Hraska V, Kostalova L, Tomeckova E. Thyroid hormone metabolism in pediatric cardiac patients treated by continuous povidone-iodine irrigation for deep sternal wound infection. Eur J Cardiothorac Surg 2002;21:1037–41.

62 Hunt JL, Sato R, Heck EL, Baxter CR. A critical evaluation of povidone-iodine absorption in thermally injured patients. J Trauma 1990;20: 127–9.

63 Steen M. Review of the use of povidone-iodine (PVP-I) in the treatment of burns. Postgrad Med J 1993;69(Suppl):S84–92.

64 Machet L, Couhé C, Perrinaud A, Hoarau C, Lorette G, Vaillant L. A high prevalence of sensitisation still persists in leg ulcer patients: a retrospective series of 106 patients tested between 2001 and 2002 and a meta analysis. Br J Dermatol 2004;150:929–35.

65 Juhász I. Experiences with the use of povidone-iodine-containing local therapeutics in dermatological surgery and in the treatment of burns: testing for allergic sensitisation in post-surgery patients. Dermatology 2002;204(Suppl):52–8.

66 Lachapelle JM. Allergic contact dermatitis from povidone-iodine: a re-evaluation study. Contact Derm 2005;52:9–10.

67 Neidner R. Cytotoxicity and sensitisation of povidone-iodine and other frequently used antiinfective agents. Dermatology 1997;195(Suppl): 89–92.

68 Dissemond J, Lehnen M, Körber A. Contact allergy in patients with chronic leg ulcers. Z Wundheil 2006;11:20–4.

69 Lyman IR, Tenery JH, Basson RP. Correlation between decrease in bacterial load and rate of wound healing. Surg Gynecol Obstet 1970;130: 616–21.

70 Hendley JO, Ashe KM. Effect of topical antimicrobial treatment on aerobic bacteria in the stratum corneum of human skin. Antimicrob Agents Chemother 1991;35:627–31.

71 Gallard RB, Saunders JH, Mosely JG, Darrell JH. Prevention of wound infection in abdominal operations by preoperative antibiotics or povidone-iodine. Lancet 1977;2:1045.

72 Viljanto J. Disinfection of surgical wounds without inhibition of wound healing. Arch Surg 1980;115: 253–6.

73 Sindelar WF, Mason GR. Intraperitoneal irrigation with povidone-iodine solution for the prevention of intra-abdominal abscesses in the bacterially contaminated abdomen. Surg Gynecol Obstet 1979;148:409–11.

74 Gray JG, Lee MJ. The effect of topical povidone iodine on wound infection following gastrointestinal surgery. Br J Surg 1981;68:310–3.

75 Walsh JA, Watts JM, McDonald PJ, Finlay-Jones JJ. The effect of topical povidone-iodine on the incidence of infection in surgical wounds. Br J Surg 1981;68:185–9.

76 Gravett A, Sterner S, Clinton JE, Ruiz E. A trial of povidone-iodine in the treatment of infection in sutured lacerations. Ann Emerg Med 1987;16: 167–71.

77 Lammers RL, Fourré M, Callaham ML, Boone T. Effect of povidone-iodine and saline soaking on bacterial counts in acute, traumatic, contaminated wounds. Ann Emerg Med 1990;19:709–14.

78 Marshall C, Queen J, Manjooran J. Honey vs povidone iodine following toenail surgery. Wounds 2005;1:10–8.

79 Edwards PG, Lipp A, Holmes A. Preoperative skin antiseptics preventing surgical wound infection after clean surgery. Cochrane Database Syst Rev 2004 (3) CD003949.

80 Kucan JO, Robson MC, Heggers JP, Ko F. Comparison of silver sulfadiazine, povidone-iodine and physiological saline in the treatment of chronic pressure ulcers. J Am Geriatr Soc 1981; 29:232–5.

81 Knutson RA, Merbitz LA, Creekmore MA, Snipes HG. Use of sugar and povidone-iodine to enhance

wound healing: five year's experience. South Med J 1981;74:1329–35.
82 Skog E, Arnesjo B, Troëng T, Gjöress JE, Bergljung L, Gundersen J, Hallböök T, Hessman Y, Hillström L, Månsson T, Eilard U, Eklöff B, Plate G, Norgren L. A randomised trial comparing cadexomer iodine and standard treatment in the out-patient management of chronic leg ulcers. Br J Dermatol 1983;109:77–83.
83 Ormiston MC, Seymour MTJ, Venn GE, Cohen RI, Fox JA. Controlled trial of Iodosorb in chronic venous leg ulcers. Br Med J 1985;291:308–10.
84 Hansson C. The effects of cadexomer iodine paste in the treatment of venous leg ulcers compared with hydrocolloid dressing and paraffin gauze dressing. Cadexomer Iodine Study Group. Int J Dermatol 1998;37:390–6.
85 Piérard-Franchimont C, Paquet P, Arrese JE, Piérard GE. Healing rate and bacterial necrotizing vasculitis in venous leg ulcers. Dermatology 1997;194: 383–7.
86 Fumal I, Braham C, Paquet P, Piérard-Franchimont C, Piérard GE. The beneficial toxicity paradox of antimicrobials in leg ulcer healing impaired by a polymicrobial flora: a proof-of-concept study. Dermatology 2002;204(Suppl):70–4.
87 Eming SA, Smola-Hess S, Kurschat P, Hirche D, Kreig T, Smola H. A novel property pf povidon-iodine: inhibition of excessive protease levels in chronic non-healing wounds. J Invest Dermatol 2006;126:2731–3.
88 O'Meara SM, Cullum NA, Majid M, Sheldon TA. Systematic review of antimicrobial agents used for chronic wounds. Br J Surg 2001;88:4–21.
89 Jones V. Use of hydrogels and iodine in diabetic foot ulcers. Diabetes Foot 1999;2:47–54.
90 Lawrence JC. A povidone-iodine medicated dressing. J Wound Care 1998;7:332–6.
91 Drosou A, Falabella A, Kirsner RS. Antiseptics on wounds: an area of controversy. Wounds 2003;15: 149–66.
92 Apelqvist J, Ragnarson Tennvall G. Cavity foot ulcers in diabetic patients: a comparative study of cadexomer iodine and standard treatment. Acta Derm Venereol 1996;76:231–5.
93 Vowden P, Cooper RA. An integrated approach to managing wound infection. EWMA position document: management of wound infection. London: MEP Ltd, 2006:2–6.
94 Eron LJ, Lipsky BA, Low DE, Nathwani D, Tice AD, Vilturo GA. Managing skin and soft tissue infections: expert panel recommendations on key decision points. J Antimicrob Chemother 2003; 52(Suppl):13–7.
95 Rodeheaver G, Bellamy W, Kody M, Spatafora G, Fitton L, Leyden K, Edlich R. Bactericidal activity and toxicity of iodine-containing solutions in wounds. Arch Surg 1982;117:181–6.
96 Oberg MS, Lindsey D. Do not put hydrogen peroxide or povidone-iodine into wounds. Am J Dis Child 1987;141:27–8.
97 Goldenheim PD. An appraisal of povidone-iodine and wound healing. Postgrad Med J 1993;69 (Suppl):S97–105.
98 Gilchrist B. Should Iodine be recognised in wound management? J Wound Care 1998;6:148–50.
99 Mayer DA, Tsapogas MJ. Povidone-iodine and wound healing: a critical review. Wounds 1993;5:14–23.
100 Burks RI. Povidone-iodine solution in wound treatment. Phys Ther 1998;78:212–8.
101 Banwell H. What is the evidence for tissue regeneration impairment when using a formulation of PVP-I antiseptic on open wounds? Dermatology 2006;12(Suppl):66–76.
102 Bergin SM, Wraight P. Silver based wound dressings and topical agents for treating diabetic foot ulcers. Cochrane Database Syst Rev 2006 (1) CD005082.
103 Vermeulen H, Ubbink DT, Goossens A, de Vos R, Legemate DA. Systematic review of dressings and topical agents for surgical wounds healing by secondary intention. Br J Surg 2005;92:665–72.

ENCLOSURE VII

ON THE USE OF CERTAIN ANTISEPTIC SUBSTANCES IN THE TREATMENT OF INFECTED WOUNDS.*

BY H. D. DAKIN, D.Sc., F.I.C.,
THE HERTER LABORATORY, NEW YORK.

IN order to make a judicious choice of the antiseptic most likely to give useful results in the treatment of infected wounds many different factors have to be considered in addition to germicidal activity, including the irritating properties of the substances, their toxicity, solubility, ability to penetrate tissues and to be absorbed, and their chemical reactions with proteins and other tissue constituents.

The killing of bacteria by ordinary antiseptic substances is essentially a chemical reaction between the antiseptic on the one hand and the proteins and other cell constituents of the micro-organism on the other. The destruction by antiseptics of bacteria suspended in water is easily effected, because no proteins are present in the mixture other than those derived from the micro-organism. The destruction by antiseptics of bacteria mixed with blood serum, pus, and other exudate is much more difficult because the antiseptic acts not only on the micro-organisms but on other protein substances as well. Therefore, in judging of the antiseptic action of a substance suitable for the treatment of wounds, it is essential that its germicidal action be tested against micro-organisms mixed with blood serum or similar substances, and not simply tested against bacteria suspended in water.

The germicidal activity of all known antiseptics is greatly reduced by the presence of blood serum or similar substances, and in some cases this reduction is so great that the compound loses all practical antiseptic value.

The following table contains results which illustrate this enormous reduction in germicidal action by blood serum in the case of several common antiseptics. I am greatly indebted to my colleague, Dr. Maurice Daufresne, for all the bacteriological results referred to in this communication.

Antiseptic.	Without Blood Serum.	With Blood Serum.
Phenol	1 : 250 − 1 : 500 +	1 : 50 − 1 : 100 +
Salicylic acid	1 : 2,500 − 1 : 5,000 +	1 : 100 − 1 : 250 +
Hydrogen peroxide ...	1 : 3,500 − 1 : 8,000 +	1 : 1,700 − 1 : 2,000 +
Iodine	1 : 100,000 − 1 : 1,000,000 +	1 : 1,000 − 1 : 2,500 +
Mercuric chloride ...	1 : 5,000,000 − 1 : 10,000,000 +	1 : 25,000 − 1 : 50,000 +
Silver nitrate	1 : 1,000,000 − 1 : 10,000,000 +	1 : 10,000 − 1 : 25,000 +
Sodium hypochlorite...	1 : 500,000 − 1 : 1,000,000 +	1 : 1,500 − 1 : 2,000 +
Benzene sodium sulphochloramide	1 : 500,000 − 1 : 1,000,000 +	1 : 1,000 − 1 : 2,000 +
Paratoluene sodium sulphochloramide	1 : 750,000 − 1 : 1,500,000 +	1 : 2,000 − 1 : 3,000 +
Acetylchloramino-dichlorbenzene	1 : 500,000 − 1 : 1,000,000 +	1 : 2,500 − 1 : 6,000 +

The figures indicate the concentration of antiseptic necessary to sterilize one drop of a fresh culture of *Staphylococcus aureus* in a total volume of 5 c.cm. acting for two hours. + indicates growth; − indicates complete sterilization.

But in choosing a suitable antiseptic many other factors than germicidal action need to be considered. Mercuric chloride, which among the substances referred to in the table shows the highest germicidal action, is probably the least useful and most objectionable as an antiseptic for the treatment of infected wounds. It may be of use to consider some of the limitations of the commonly used substances referred to in the above table.

Phenol is characterized by very low germicidal power, especially when acting in the presence of serum. When used in sufficiently high concentration for germicidal efficiency it is decidedly destructive of healthy tissue.

Hydrogen peroxide gives encouraging results when tested against bacteria in the test tube, but when used on wounds the substance has little germicidal action, for it is decomposed with the greatest ease by the enzyme catalase present in all tissues and in the blood cells. Hence its action can only be exerted during a trifling interval of time. The mechanical detergent action connected with the rapid disengagement of oxygen gas on infected surfaces is probably of greater value than any antiseptic action exerted by the hydrogen peroxide.

An interesting experiment related to me by Professor E. K. Dunham may be quoted here. A rabbit which had received an intravenous injection of the Welch bacillus (*B. aërogenes capsulatus* or *B. perfringens*) was killed, and the infected liver was removed and carefully sectioned. It was found that cubes of the infected liver only 1 mm. in size could be immersed in and incubated with hydrogen peroxide of moderate concentration without destruction of the micro-organisms.

Hydrogen peroxide, as regards its antiseptic action, must be regarded as of slight value, even against anaërobic organisms.

Mercuric chloride readily loses most of its antiseptic action in presence of many tissue constituents, and, as is well known, is irritating even in dilute solution. It is useless for the sterilization of pus when employed at any reasonable concentration.

Silver nitrate is of greater value than mercuric chloride, but when used in sufficiently high concentration is irritating. Many tissue constituents inhibit its action markedly. The photo-sensitiveness of the silver compounds formed is objectionable.

Iodine, which has proved so valuable for the disinfection of skin, has given much less satisfactory results when used for deep wounds owing to protein coagulation and irritation of the tissues. The penetrating power of iodine is slight, and wounds which have been freely treated with it are apt to cicatrize more slowly than others.

Sodium hypochlorite has high germicidal action, and has many other desirable properties. But sodium hypochlorite as ordinarily prepared is of extremely variable composition, contains free alkali and sometimes free chlorine, and is consequently irritating when applied to wounds. By a simple process, which will now be described, it has been possible to render the hypochlorites much less irritating while retaining their antiseptic action unchanged.

PRINCIPLES INVOLVED IN THE PREPARATION OF THE HYPOCHLORITE SOLUTION.

Solutions of sodium hypochlorite always contain free alkali even when prepared with the greatest care. A so-called "neutral" solution of sodium hypochlorite has an alkaline reaction. This is due not only to free alkali which may remain from the process of preparation, but also to the fact that the hypochlorite in solution undergoes hydrolytic dissociation giving free sodium hydroxide and hypochlorous acid.

$$NaClO + H_2O = NaOH + HClO$$

The extent of this dissociation has been measured by Duyk, and quantitatively it is very considerable. The irritating action of ordinary hypochlorites is largely due to this formation of free alkali. The extent of this hydrolytic dissociation increases with dilution, so that practically hypochlorites cannot be effectively rendered non-irritating by simply reducing the concentration, for a point is soon reached at which germicidal action is impaired while the irritating properties of the solution persist. In addition to the above sources of free alkali, it must not be forgotten that alkali may be liberated by the action of sodium hypochlorite on proteins, a reaction in which the chlorine of the hypochlorite is attached to nitrogen in the proteins, as will be shown later.

Now it is well known that certain fluids, such as blood and some other body fluids, also certain artificial salt solutions containing mixture of salts of polybasic acids—for example, phosphoric acid—are able to retain their essential neutrality even after the addition of limited quantities of acid or alkali. This is due to the fact that the addition of acid or alkali simply changes the relative

* The work described in this communication was carried out in laboratories at Compiègne supported by the Rockefeller Institute for Medical Research attached to Hospital 21 of the French army. For cordial co-operation in the preparation of a large number of chloramines and other substances, upon which a detailed report will be published later, I am indebted to my former teacher, Professor J. B. Cohen, F.R.S., of the University of Leeds, and to Dr. J. Kenyon, who was appointed by the British Medical Research Committee.

proportion of two or more salts of the polybasic acid present in the solution.

Starting with this idea, and employing the feeble polybasic acid, boric acid, it has been possible to prepare a simple hypochlorite mixture which maintains approximate neutrality under all conditions, is practically non-irritating, and which, when properly applied, has given most encouraging results in the antiseptic treatment of wounds. It must be understood that the insignificant antiseptic action of boric acid has nothing to do with the employment of this acid; nor is the boric acid employed for the purpose of liberating hypochlorous acid, as in Lumière's or Lorrain Smith's preparations.

The principle of the preparation is as follows: Chloride of lime (bleaching powder) is decomposed with a solution of sodium carbonate and the filtered solution containing sodium hypochlorite together with a slight excess of alkali is mixed with boric acid in such quantity that the solution is acid to phenolphthalein suspended in water but still alkaline to litmus. The resultant solution contains a balanced mixture of hypochlorite and polyborates of sodium with small amounts of free hypochlorous and boric acids. Thus the irritating action of free caustic alkali is avoided, for even if momentarily formed it would be at once neutralized by the boric acid or acid borates present in the solution.

Preparation of Solutions.

The preparation of a solution of suitable concentration for direct application, containing 0.5 to 0.6 per cent. of sodium hypochlorite, may be carried out very simply as follows:

One hundred and forty grams of *dry* sodium carbonate (Na_2CO_3), or 400 grams of the crystallized salt (washing soda), is dissolved in 10 litres of tap water, and 200 grams of chloride of lime (chlorinated lime) of good quality is added. The mixture is well shaken, and, after half an hour, the clear liquid is siphoned off from the precipitate of calcium carbonate and filtered through a plug of cotton; 40 grams of boric acid are added to the clear filtrate, and the resulting solution is ready for use. A slight additional precipitate of calcium salts may slowly occur, but it is of no significance. The solution should not be kept longer than one week. *The boric acid must not be added to the mixture before filtering, but afterwards.*

A stronger solution may be prepared by decomposing chloride of lime with sodium carbonate in the proportion of 150 grams of the former to 105 grams of the latter dissolved in a litre of water. The mixture is filtered and a measured portion of it (20 c.cm.) is rapidly titrated with a boric acid solution of known strength (31 grams per litre), using phenolphthalein suspended in water as indicator, in order to determine the amount of solid boric acid to be added to the rest of the filtrate. An excess of boric acid should be avoided, so that it is best to add slightly less than the calculated amount. An ordinary alcoholic solution of phenolphthalein cannot be used as indicator, as the alcohol is at once attacked.

The concentrated solution thus prepared contains about 4 per cent. of sodium hypochlorite, and should be mixed with six parts of water before use. It can be kept for a month without serious decomposition. Such a solution is now prepared by Poulenc Frères, 122, Boulevard St. Germain, Paris, but it can easily be made at a negligible cost by any competent chemist, and I hope that it may be so made generally.

APPLICATION AND RESULTS.

To obtain the best results it is essential to commence the antiseptic treatment of the wound at the earliest moment possible, and to bring fresh quantities of the antiseptic solution in contact with all parts of the wound as frequently as possible for a considerable period of time. This is naturally a difficult problem, requiring different methods for various types of wound. The methods of applying the solution which have been found useful at Compiègne will be described by Dr. Carrol. But to give some idea of the quantities of solution employed it may be mentioned that 5 to 10 c.cm. may be introduced every two hours by means of rubber tubes into small wounds, using a pipette or syringe, while for the irrigation of such wounds as fractured femurs, accompanied by much destruction of tissue, as much as 1, or even 2, litres a day may be employed. The dilute solution, prepared as described, may be used in large quantities for the continued irrigation or instillation of wounds for more than a week without producing visible irritation. It is extremely rare for slight irritation of the skin to occur, and this may be guarded against by the application of vaseline to the skin adjacent to the wound. As a wet dressing the solution may be used almost indefinitely. A few comparative tests on similar surface wounds do not indicate that cicatrization is delayed, even by its continued use.

The solution has the valuable property of assisting in the rapid dissolution of necrosed tissue, this being doubtless due to the ability of hypochlorites to attack the (NH) groups present in proteins with formation of soluble products. It has a certain haemostatic action as well but is actively haemolytic, and should not be injected intravenously.

It is difficult in a printed communication to produce simple convincing evidence of the usefulness of an antiseptic. Records of a few individual cases treated with brilliant results are, of course, of no great value, for many infected wounds do well with a minimum amount of treatment, but the clinical results obtained during six months' use of the solution by a number of observers in different hospitals warrant the belief that the solution is of genuine value. By far the most striking results are seen in ambulances, where treatment can be commenced a few hours after the wound has been received. Among these cases the proportion of cases which at no time show a significant rise in temperature and in which healing without suppuration occurs is very large. In many cases it has been possible to make comparative tests, with and without antiseptic, on similar wounds with striking results. Records obtained by means of serial coloured photographs of the gradual changes in wounds of the most varied kind under different conditions show definite differences in favour of the solution, and in no case has any objectionable after-effect been traced to the action of the antiseptic. It should be stated that most of the cases treated with the antiseptic were kept under observation for several weeks until discharged as convalescent. This is, of course, important for judging of the ultimate value of the treatment.

An idea of the antiseptic properties of the solution may be gathered from the following figures: Staphylococci suspended in water are killed in two hours at a concentration of hypochlorite between 1 : 500,000 and 1 : 1,000,000, while in the presence of serum the necessary concentration is between 1 : 1,500 and 1 : 2,000. Streptococci are more readily killed, while *pyocyaneus* suspended in water is killed in two hours at a concentration between 1 : 100,000 and 1 : 1,000,000, while in serum between 1 : 2,500 and 1 : 5,000 is necessary.

Hypochlorites are extremely active substances chemically, and they should not be used in conjunction with other antiseptics nor with alcohol or ether. Wounds which have been previously treated with much iodine may take on a dark colour, due to the re-liberation of iodine, but this is of no importance.

Many other preparations of hypochlorites have been employed at various times by different workers. The more commonly recommended preparations are the ordinary alkaline solutions of the hypochlorites of sodium, potassium (eau de Javelle), or calcium; while mixtures of powdered chloride of lime with boric acid have been employed by Vincent, Lumière, and by Lorrain Smith and others. It is believed that the solution previously described, when properly applied to all parts of the wound, gives better results than can possibly be obtained from powdered preparations of partially soluble materials. The local production of hypochlorites, hypochlorous acid, or chlorine in high concentration, such as results from the use of the powdered mixture, is much more dangerous for healthy tissue than is the continued application of a weak neutral solution of sodium hypochlorite. Generally speaking, our experiments with powdered substances have given much less good clinical results than have aqueous solutions. It is true, however, that aqueous solutions need more care for their successful application, for it is essential that they reach every part of the infected area, and that the antiseptic should be renewed from time to time.

MODE OF ACTION OF HYPOCHLORITES.

When a solution of a hypochlorite or of free hypochlorous acid acts upon organic substances containing the

=NH group the first reaction almost always consists in the replacement of hydrogen by chlorine with formation of substances of the group known as chloramines. All protein substances contain an abundance of these groups, and they readily react with hypochlorites:

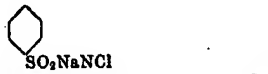
$$\begin{matrix} R^1 \\ R^2 \end{matrix}\!\!>\!NH + HClO = \begin{matrix} R^1 \\ R_2 \end{matrix}\!\!>\!NCl + H_2O$$

The antiseptic action of hypochlorites doubtless depends upon reactions of this type. It was therefore interesting to examine many different varieties of the large group of chloramines in order to study their antiseptic actions. In this work I have enjoyed the co-operation of Professor J. B. Cohen of the University of Leeds.

In the first place, it may be stated that all substances containing the =NCl group were found to be strongly antiseptic, and some of them will probably be found to have practical value. Proteins, such as blood serum, egg white, casein, etc., when treated with hypochlorites, give products of high antiseptic value, and undoubtedly compounds of this type are formed *in situ* when wounds are treated with hypochlorites. This is doubtless an advantage, as in this way a certain antiseptic action may be expected to persist even after the free hypochlorite has disappeared.

Substances such as acetanilide when treated with hypochlorous acid under appropriate conditions, carefully studied by Chattaway, give chloramines—for example, acetylchloraminodichlorbenzene — which are sparingly soluble in water, but which may be dissolved in vaseline or lanoline. Although the germicidal power of these compounds is very high indeed, the action on infected wounds of strong solutions of them in vaseline or lanoline was not markedly superior to that of plain vaseline. It appears that, generally speaking, active germicidal action can hardly be hoped for from sparingly soluble antiseptics mixed with fatty substances. Anaërobic organisms can readily grow under the fatty film covering the surface of the infected area.

On the other hand, certain aromatic chloramines which form soluble sodium salts have given most encouraging clinical results. The best of these compounds are the benzene or paratoluene sodium sulphochloramides, both of which have been described by Chattaway.



Benzene sodium sulphochloramide.

Paratoluene sodium sulphochloramide.

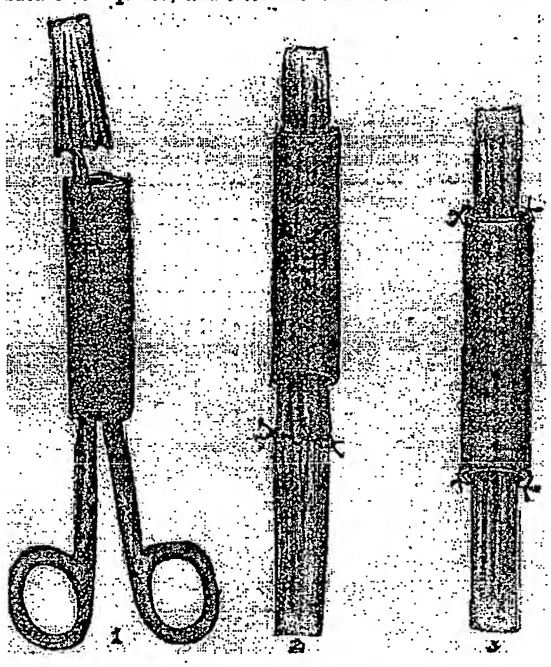
These substances are extremely powerful antiseptics, are practically non-irritating, and can be used in much higher concentration than can the hypochlorites. A 2 to 4 per cent. solution may be conveniently employed. In general, the action of these substances is similar to that of the hypochlorites, but more powerfully antiseptic. They have, however, no special solvent action on necrosed tissue, this being doubtless due to the fact that the active chlorine in these compounds is already attached to nitrogen. While the number of cases thus far treated with these antiseptics is smaller than those treated with the hypochlorite mixture, excellent results have been obtained in a number of badly infected wounds, notably compound fractures of the femur. It appears probable that these chloramides, which are relatively easily prepared at low cost, and which have the advantage of being stable solids, may be found useful for other purposes than for the treatment of infected wounds. Their possible applications will be the subject of further study.

Benzene sodium sulphochloramide kills staphylococci suspended in water in two hours at a concentration of 1 : 500,000, and the toluene derivative kills at 1 : 1,000,000. In the presence of serum the necessary concentrations are about 1 : 1,500 and 1 : 2,500 respectively. *Bacillus pyocyaneus*, *B. typhosus*, and *B. coli* are slightly more resistant than staphylococci, while *B. aërogenes capsulatus* and streptococci are more readily killed. The concentrations refer to the weight of the crystallized salts. It will be seen that the molecular concentration of toluene sodium sulphochloramide necessary to kill staphylococci in the presence of serum is only about one-fifth of the correspondingly active molecular concentration of sodium hypochlorite.

ON THE USE OF A SLEEVE OF VEIN IN NERVE SUTURE.

By ANDREW FULLERTON, M.Ch., F.R.C.S.Irel.,
Colonel (Temporary) A.M.S.; Consulting Surgeon to the Forces in France; Surgeon in Charge of Out-patients, Royal Victoria Hospital; Surgeon, Ulster Volunteer Force Hospital.

During the present war many cases of nerve injury have been recorded. The injuries are produced for the most part by rifle bullets, fragments of shell, and shrapnel. Primary suture is frequently out of the question, and the wounds are allowed to heal without any attempt being made to suture the divided nerves. Later, secondary suture is required, and often the divided ends have to be



sought for in a large amount of scar tissue. It is essential in cases of this sort to protect the junction so as to avoid ingrowing of scar tissue between the nerve ends, and consequent failure of the operation. To prevent this various substances have been used, including decalcified bone tubes, gelatine tubes, animal's artery, paraffin wax, Cargile membrane,* and human vein. Sherren (*Injuries of Nerves*) prefers chromicized Cargile membrane.

For some years I have been using portions of vein in the manner here illustrated. The most suitable vein for nerves of the upper extremity—as, for instance, the musculo-spiral, the median, and the ulnar—is the basilic vein at a spot between its commencement and the point at which it pierces the deep fascia of the upper arm. A segment of the vein about 1½ in. or 2 in. in length is excised and threaded on a sinus forceps as in Fig. 1. One end of the nerve is then caught by the forceps, and the sleeve pulled over as in Fig. 2. The ends of the nerve are then freshened with a sharp scalpel and sutured with fine catgut. When the suture is complete the sleeve is pulled over the junction, as in Fig. 3, and fastened to the nerve sheath by a few points of suture. The vein thus applied is intended to form an aseptic sheath for the nerve, to keep the ends in secure apposition, to direct the growth of the new axis cylinders, and to prevent the ingrowth of scar tissue from the outside. Any vein of suitable size will, of course, do, and in the lower extremity a portion of the internal or external saphenous will probably be the most convenient.

The sleeve must be pulled over the first nerve end before trimming so as to avoid damage to the freshly cut end.

Possibly this method has been in use by others, but I have not seen it used or described up to the present.

* Peritoneum of the ox.

ENCLOSURE VIII

Library (Scientific Research) Section

President F C Kelly PhD

Meeting June 14 1961

President's Address

Iodine in Medicine and Pharmacy Since its Discovery—1811-1961

by Francis C Kelly BSc PhD FRIC (*London*)

To compress into one brief hour the whole medical history of iodine since its discovery by the French chemist, Bernard Courtois, one hundred and fifty years ago, is manifestly impossible. I propose, therefore, to follow only one or two of the more important threads that make-up the warp and woof of what for me has always been a fascinating story.

Like so many other scientific advances, the discovery of iodine was a discovery quickened under the urgency and pressure of war. At the beginning of the nineteenth century, France, obedient to the dictates of Napoleon's ambitious dreams, was fighting with almost all her neighbours and she needed enormous quantities of gunpowder to do the job. Hemmed-in on land by the armies of Prussia and Austria, and blockaded at sea by the British Navy, her supplies of chemicals necessary for the manufacture of gunpowder were cut off. One of these chemical essentials was saltpetre – nitrate. What did France do? As she was unable to get nitrate from beyond her borders, she made it at home, artificially, in specially constructed nitre beds. And it was while engaged in the manufacture of saltpetre on one of these artificial 'nitre plantations', near Paris, that Bernard Courtois made his discovery.

The story has often been told. The key-point is that to make saltpetre you must have a plentiful supply of soda. Courtois obtained his soda from the ashes of seaweed – called variously *varec*, *varech*, or *vareck* in French, and *wrack* or, more commonly, *kelp* in English. Extraction of the soluble sodium carbonate from the seaweed ash was carried out in metal vats, and after successive extractions there remained at the bottom of the vessels a thick mother liquor encrusted with insoluble material.

From time to time it was necessary to clean out these deposits with the aid of acid and heat. It seems that one day Courtois added stronger acid than usual, for he was astonished to see intensely beautiful violet-coloured vapours arising from the mixture. He noticed, furthermore, that these vapours formed a deposit of lustrous metal-like crystals on the cooler parts of the vessel.

Here was a new and strange phenomenon. His curiosity aroused, Courtois scraped off some of the mysterious substance, and, by a brief examination, determined its more obvious chemical properties. But he was far too busy with the management of his saltpetre factory to carry his investigations very far; so he gave specimens of the new product to his closest professional friends asking them to continue the study where he had left off. Among those who received specimens were Gay-Lussac, the most distinguished French chemist of his day, and also Ampère.

Now, all this happened towards the end of the year 1811 – probably in the month of November – the exact date of the discovery of iodine is not known.

Two years elapsed.

On October 27, 1813, there arrived in Paris from England a remarkable quartette. They were: Sir Humphry Davy, the eminent English chemist; his temperamentally unpredictable wife, Lady Davy; her lady's maid; and Davy's assistant and valet, none other than Michael Faraday, then an obscure young man of 22, who was eventually to become more famous than his master.

Remember that England was then at war with France; but in those days wars were solely the affair of soldiers and sailors; men of science continued to correspond with each other and remained constantly on good terms. So it was that when Davy expressed a desire to pass through France on a journey to Italy, Napoleon, always an ardent patron of science, immediately ordered that passports be issued.

Among the first to greet Davy on his arrival in Paris was Ampère who, in an eager and generous moment, gave Davy a sample of the unknown 'X', as they called it. Davy got to work upon it and soon satisfied himself that it was a new elementary

substance analogous to chlorine. On tour, Davy always carried a compact travelling laboratory around with him – a 'chemistry set' we would call it nowadays.

Gay-Lussac – Davy's only professional equal in France, or indeed anywhere else – was furious when he heard what Ampère had done. His irritation is understandable. He was annoyed, not so much with Ampère, as with himself for being so slow. After all, he had had substance 'X' in his hands for two years and had already proved its elementary nature; but alas, had published nothing.

There followed an undignified scramble into print to establish priority, and a bitter 'priority' quarrel broke out between Davy and Gay-Lussac. The details need not detain us; they are well documented.

For posterity, the happy outcome of these controversial days was the birth of a new and beautiful element. Its name, given by Gay-Lussac in French – 'iode' – and anglicized by Davy – 'iodine' – is derived, like so many of the loveliest words in English, from the Greek – from the word ἰοειδής meaning 'violet coloured'.

From Folk Medicine to Rational Therapy

Comparatively soon after its discovery, iodine found favour as a therapeutic agent – a medicine.

Looking backwards from the precarious heights of 1961 down through the past century and a half, we can discern two distinct lines of advance which could not, of course, be foreseen by those who launched iodine upon its medical and pharmaceutical career.

The first illustrates perfectly how, as medical science progresses, the unexplained virtues of a traditional remedy – some part of folk medicine, let us say – become wholly intelligible when exposed to the light of exact biological knowledge.

In 1656, more than one hundred and fifty years before the discovery of iodine, a London physician called Thomas Wharton described and named a gland in the neck: he called it the *thyroid* gland – the shield-shaped gland. He says: 'It contributes much to the beauty of the neck, filling-up the vacant spaces around the larynx, making its protuberant parts smooth, particularly in females, to whom for this reason a larger gland has been assigned, which renders their necks more even, and more beautiful.' Unhappily, thyroid glands do not always retain these delicate proportions. They sometimes get out of gear inside, and grow into the large unsightly swellings that we describe under the general term of goitre.

Goitre has troubled mankind from time immemorial; and from time immemorial man has sought to overcome it. Centuries before the discovery of iodine, the most highly-prized remedy for goitre was a concoction containing the ashes of burnt sea sponge. Some say that this remedy originated in the very early medical experience of China, where goitre then abounded and indeed still abounds. Certainly the burnt-sponge remedy was known to European medicine in the Middle Ages (thirteenth century, let us say) and eventually found its way to England, where, in the mid-1700s, burnt sponge was famed as the 'Coventry Remedy'.

For many a year, the Coventry recipe was held secret by the family of a Dr Bate of that town. They administered it with such success as to gain them a wide reputation and no small fortune. Only in 1779 was it disclosed that the essential ingredient was burnt sponge.

Now, in 1819, eight years after the discovery of iodine, Andrew Fyfe, then lecturer in chemistry at the University of Edinburgh, found that the common sea sponge always contains exceptionally high quantities of iodine. Although Fyfe did not realize it, his discovery was important because it provided a link in the chain of evidence that proved iodine to be the active curative principle in burnt sponge.

In the same year (1819) a prominent physician in Geneva, Jean François Coindet, ignorant of Fyfe's findings, but aware of the virtues of burnt sponge and of seaweed ash as remedies for goitre, suspected that iodine, discovered in seaweed but a few years earlier, might be the active therapeutic agent common to these two marine products.

Coindet's test of this hypothesis met with dramatic success. By giving tincture of iodine to goitre patients – there were plenty of them in Switzerland, his native country – he could appreciably reduce large goitrous swellings within a week. So spectacular were his results, that everywhere they gained a wide publicity, and Coindet is generally regarded as having been the first to introduce iodine into medical practice in this way.

Claims for this honour have also been advanced by the celebrated English physician and chemist, William Prout, who stated (in 1834) that he first employed potassium iodide as a remedy for goitre in 1816. He says, also, that he told Dr John Elliotson about it, and that Elliotson commonly prescribed potassium iodide at St Thomas's Hospital as early as 1819.

Although these claims cannot be verified in any contemporary record, there is no reason to doubt them. Incidentally, Dr Elliotson was the first President of this Society after its incorporation as the Royal Medical and Chirurgical Society of London in 1834; his name appears in our first Charter, and there is a good portrait of him in the Council chamber on the 4th floor.

Hard on the heels of Coindet's remarkable therapeutic results came the suggestion that the cause of goitre is a lack of iodine in the system

brought about by a deficiency of the element in water, soil, and food. Credit for this belongs to the French chemist, Gaspard Adolphe Chatin who, between the years 1850 and 1876, found that the iodine content of waters, and of foodstuffs of vegetable origin grown in goitre areas, was less than that in healthy areas.

Unfortunately, Chatin's conclusions failed to convince his contemporaries, and his attempt to establish a causal relationship between environmental iodine deficiency and the occurrence of endemic goitre lay neglected and forgotten until the end of the nineteenth century.

To-day, the opinions of Coindet and of Chatin stand completely justified. First step in their vindication was the fundamental discovery in 1895, by the German chemist Baumann, that iodine is an invariable constituent of the normal thyroid gland. This at once gave point to the iodine treatment of goitre introduced by Coindet seventy-five years earlier. Next major advance resulted from investigations carried out between the years 1913 and 1919 by E C Kendall at the Mayo Foundation, Rochester, N.Y., who was the first to isolate the iodine-containing hormone of the thyroid gland in crystalline form. He named it 'thyroxine'. Then came the notable work of Sir Charles Harington, around the years 1925 to 1930, who determined the exact chemical constitution of thyroxine, proved that the molecule contains four atoms of iodine, devised means for its artificial synthesis, and drew attention to the principal chemical features responsible for its specific physiological activity.

'It is interesting to reflect', writes Sir Charles, 'that even if we date the first therapeutic use of burnt sponge from the middle of the thirteenth century, this remedy was known as a specific for thyroid disease some five hundred and fifty years before the discovery of the element to which it owed its activity, and some six hundred and fifty years before the final demonstration of the role of the element in the economy of the thyroid gland itself.'

This gradually unfolding knowledge brought a new outlook on the whole question of local iodine deficiency as a cause of goitre. Soils, foods, and drinking-waters from goitrous and non-goitrous regions have been examined afresh by refined modern techniques, and the results amply confirm Chatin's dictum that where iodine is scarce – either in absolute or relative terms – there also will you find goitre.

Through the enlightened efforts of the World Health Organization goitre surveys have been carried out all over the world in recent years and we now have 18 major countries where, by law, all kitchen and table salt is iodized in the interests of public health. Twenty others, hesitant to impose legal compulsion, nevertheless officially encourage the community use of iodized salt as a preventive measure in the public interest.

After all, according to best estimates, there are in the world to-day 200 million people who suffer from thyroid deficiency in greater or lesser degree.

I would mention here that iodization of salt to prevent goitre is no new-fangled notion. It was first recommended one hundred and thirty years ago, in 1831, by that delightful character, J B Boussingault – mining engineer, chemist, agronomist, traveller, *bon vivant* – probably the most peripatetic scientific Frenchman of his day who, on his travels in South America, noticed that the natural salt deposits, instinctively preferred by the peoples of goitrous districts in Colombia, contained most iodine.

General Therapy

But enough of iodine-deficiency goitre; let us move to another chapter in the iodine story – the ups and downs in its career as a general therapeutic agent, and as antiseptic and disinfectant.

Around the time iodine was discovered, French medicine was in the ascendant; traditional methods – bleeding, purging, the leech – were on the way out and yielding place to the first beginnings of experimental pharmacology. Pioneer in this new movement was François Magendie then at the height of his fame as an experimentalist. He it was who first put iodine into a pharmacopœia – in the year 1821.

In the first flush of enthusiasm for the newcomer, physicians and surgeons tested it and tried it for every conceivable pathological condition. The variety of diseases for which iodine was prescribed in the early years is astonishing – paralysis, chorea, scrofula, lacrimal fistula, deafness, distortions of the spine, hip-joint disease, syphilis, acute inflammation, gout, gangrene, dropsy, carbuncles, whitlow, chilblains, burns, scalds, lupus, croup, catarrh, asthma, ulcers, and bronchitis – to mention only a few.

Indeed, tincture of iodine, iodoform, or one of the iodides, was applied to almost every case that resisted the ordinary routine of practice; and between 1820 and 1840 there appeared a remarkable series of essays and monographs testifying to the extraordinary benefits to be achieved by this new and potent remedy.

In the Great Exhibition at the Crystal Palace in Hyde Park in May 1851 iodine and iodine compounds were publicly shown for the first time by ten pharmaceutical firms, including Hopkin and Williams of London, and Howards of Ilford – both still going strong.

Iodine preparations were urged and accepted into the prescribers' manuals of the day in rapidly increasing numbers. The first British Pharma-

copoeia (1864) made a rigid selection of 14 preparations; by 1890, to choose a date at random, the 6th edition of Martindale's Extra Pharmacopoeia sponsored 30 medicaments derived from iodine; the 'Iodine Centenary Volume' compiled by 'The Prescriber' in 1914 mentions 45 iodine preparations; by 1928 'Martindale' had extended its coverage to 128 iodine items; and, in an International Index published in 1956, and devoted exclusively to iodine pharmaceuticals, no less than 1,700 approved pharmacopoeial names, proprietary names, synonyms, and alternative designations are alphabetically listed.

In the beginning, methods of applying iodine were scarcely less numerous than the variety of preparations available. The element was administered as vapour; as nascent iodine; in baths; in pills; in sweets; by inhalations; by irrigation, ionization and electrophoresis; in cigarettes; in tobacco, soaps, snuff, medicated matches, ointments, creams, hair-tonics, dusting-powders, suppositories, plasters, contraceptives, and even in an abortifacient.

Pharmaceutical literature of the nineteenth century is studded with iodine eponyms – Bryant's sherry; Churchill's caustic; Lugol's solution; Mandl's paint; Morton's fluid; Nourry's wine; Vanier's syrup; Whitehead's varnish – and so on. These men gave their names to the particular iodine preparations they invented and found useful. In French hospitals it was common practice to hang-up, on the crossbeams of the wards, strips of gauze steeped in iodoform (for all the world like old-fashioned fly-papers); and, in one Paris infirmary, the ward floors were strewn with dried seaweed in the belief that iodine emanations would pervade the atmosphere with beneficial effect.

Within a very short time of iodine's entry into medicine around 1820, ordinary people like ourselves, doubtless encouraged by the patent medicine vendors of the day, took to carrying little bottles of iodine hung round their necks, like amulets to charm away disease. Later, this practice fell into disrepute; but, oddly enough, was revived even in our own day.

Antiseptic and Disinfectant

First specific reference to the use of tincture of iodine in wounds appears to be that made in 1839 by John Davies, Surgeon to the General Infirmary, Hertford, whose 'Textbook on Surgery' included a special section on the application of tincture of iodine to lacerated, contused and punctured wounds. He did this, he says, 'to bring into general notice a remedy whose superior curative properties, as an external application, appear to be but little known to the Profession'.

The earliest recorded account of iodine tincture being applied to war wounds – wounds sustained on the battle-field – relates to the American Civil War.

On September 29, 1862, Colonel John B Gordon held the centre of General Lee's army at the battle of Antietam, or Sharpsburg. The first volley from the northern lines sent a ball through the calf of Gordon's right leg; soon after, another went through the muscles of his thigh; a third pierced his left arm, tearing asunder the tendons and mangling the flesh; a fourth ripped through his shoulder leaving a wad of clothing embedded in its track. Still, no bones were broken; but, while Gordon lingered in the firing line, 'with', as he says himself, 'but little of my usual strength', a fifth ball struck him squarely in the face.

Dr Weatherly of the 6th Alabama Regiment, in charge of medical arrangements, had the Colonel removed to a base hospital, and prescribed tincture of iodine to be painted on the wounds three or four times a day. The case was unpromising. Gordon's eyelids were greatly swollen; one eye was completely closed, the other almost so; his jaw was immovably clenched, and, to make matters worse, erysipelas had set in on the left arm.

Mrs Gordon, his wife, who nursed him – her name was Fanny, and she was then a beautiful girl of 25 – put a liberal interpretation on her instructions and painted the wounds, not three or four times a day, but, as Gordon himself says: 'I think three or four hundred times a day.' Fanny's diligence and devotion were rewarded. Her husband survived, outlived the war, became Governor of Georgia, a General, and Commander-in-Chief of the United Confederate Veterans. He died in 1904.

And so it came about that in every war throughout the remainder of the century, and indeed until the end of the 1914–1918 war, iodine tincture was declared a requisite in all field hospital stores and pharmacy waggons. Those of you who are old enough will remember the 'first field dressing' of 1914–1918, tucked into a little pocket on the left-hand inner-side corner of the battle tunic. It may fairly be said that at the beginning of the First World War most surgeons felt secure in the belief that a prompt application of a solution of iodine in spirit would suffice for the primary disinfection of a wound of moderate size; and that was why a small capsule of 2% tincture of iodine was included along with gauze and safety-pins in the 'first field dressing'.

Unhappily, the field conditions under which that war was waged inevitably gave rise to grossly infected wounds against which no chemical antiseptic was of any avail whatever, unless applied in excessive strength damaging to the living tissue. The technical reasons for this failure of iodine to

SOME LANDMARKS IN THE MEDICAL HISTORY OF IODINE

IODINE IN THE BRITISH PHARMACOPOEIA

MONOGRAPHS	1st 1864	2nd 1867	3rd 1885	4th 1898	5th 1914	6th 1932	7th 1948	8th 1953	9th 1958	Addm. 1960
ARSENIC IODIDE										Dermatology
CADMIUM IODIDE										Dermatology
HYDRIODIC ACID										Expectorant
IODINE										The Parent Substance
AQUEOUS IODINE SOLUTION										Antiseptic and Disinfectant
SIMPLE IODINE SOLUTION										Anti-Rheumatic
STRONG IODINE SOLUTION										Antiseptic and Disinfectant
WEAK IODINE SOLUTION										Antiseptic and Disinfectant
IODINE OINTMENT										Counter Irritant
IODINE VAPOUR										Respiratory Disease
IRON IODIDE										Tonic and Alterative
IRON IODIDE SYRUP										Tonic
LEAD IODIDE										Dermatology
MERCURY IODIDE, GREEN										Antisyphilitic
MERCURY IODIDE, RED										Antisyphilitic
POTASSIUM IODIDE										Alterative, Antisyphilitic, Expectorant, Diuretic, Etc.
SODIUM IODIDE										Alterative, Antisyphilitic, Etc.
SULPHUR IODIDE										Dermatology
CHINIOFON	Amoebicide									
DECAMETHONIUM IODIDE	Anaesthesia									
DI-IODOHYDROXYQUINOLINE	Amoebicide									
DIODONE	Contrast Medium									
EMETINE BISMUTH IODIDE	Amoebicide									
GALLAMINE TRIETHIODIDE	Anaesthesia									
IODOFORM	Antiseptic									
IODOPHTHALEIN	Contrast Medium									
IODOXYL	Contrast Medium									
IOPANOIC ACID	Contrast Medium									
LIOTHYRONINE, SODIUM	Thyroid Hormone									
PHENIODOL	Contrast Medium									
PROPYLIODONE	Contrast Medium									
THYROID, DRIED	Thyroid Hormone									
THYROXINE, SODIUM	Thyroid Hormone									

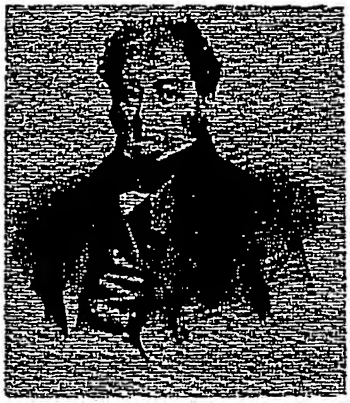
Table 1

FROM FOLK MEDICINE
to
RATIONAL THERAPY

4th to 8th Century A.D.	INFUSION OF SEAWEED used to treat thyroid enlargement (goitre) by Chinese physician KE-HUNG (281-361). WANG T'AO (8th Century) lists 36 goitre prescriptions, 27 of which contain preparations of seaweed or other marine product.
1779	COVENTRY REMEDY. A secret remedy for goitre, now known to have contained "calcined spunge" brought a wide reputation and no small fortune to its owners, a doctor and an apothecary in Coventry. Details were published in 1779..
1811	DISCOVERY OF "A NEW SUBSTANCE" in seaweed ash by BERNARD COURTOIS (1777-1838). Not announced until 1813.
1813 27 Oct.	ARRIVAL of HUMPHRY DAVY (1778-1829) in Paris *en route* to Italy. Given a sample of the new substance; submitted it to experiment and "soon satisfied himself that it was a new elementary body analogous to chlorine."
1813 29 Nov.	FIRST PUBLIC ANNOUNCEMENT of COURTOIS' discovery made by C. B. DESORMES (1777-1862) and N. CLÉMENT (1779-1841) at meeting of Imperial Institute of France.
1813 6 Dec.	NAMING OF IODINE. J. L. GAY-LUSSAC (1778-1850) made known the results of *his* preliminary experiments, claimed the substance to be a new element and named it *Iode.*
1813 10 & 11 Dec.	CONTROVERSY. From Paris, HUMPHRY DAVY sent communications to The Royal Society of London and to the Imperial Institute of France seeking to establish that he, not GAY-LUSSAC, was the the first to prove iodine an element.
1816	FIRST MEDICAL USE? Writing in 1834, WILLIAM PROUT, M.D., F.R.S. (1785-1850) claims that he first used potassium iodide as a remedy for goitre in 1816.
1816	DISCOVERY IN SPONGES. ANDREW FYFE, M.D. (1792-1861), lecturer in chemistry, Edinburgh University and later professor of medicine and of chemistry at King's College, Aberdeen, demonstrated that iodine is a normal constituent of the common sponge.
1819	TINCTURE A GOITRE SPECIFIC. J. F. COINDET, M.D. (1774-1834) of Geneva, announced in 1820 to the Swiss Society of Natural Sciences his discovery, a year previously, that iodine tincture is a specific for goitre. COINDET is generally regarded as having been the first to introduce iodine into medical practice.

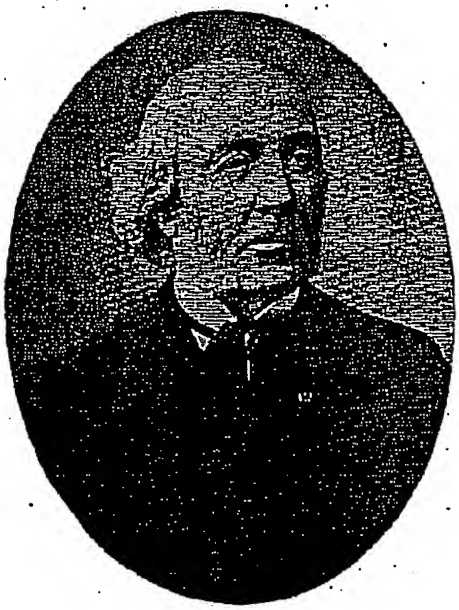
1819	SAINT THOMAS'S HOSPITAL. JOHN ELLIOTSON*, M.D., F.R.S. (1786-1868). Adopted potassium iodide as a remedy for goitre at St. Thomas's Hospital on the suggestion of Prout (*q.v.* 1816).
1831	IODIZED SALT FIRST RECOMMENDED. J. B. BOUSSINGAULT (1802-1887) noticed in 1825 that local salt supplies instinctively preferred by the inhabitants of goitrous districts in Colombia contained most iodine. He therefore recommended the iodization of cooking salt for goitre prevention.
1838	VIRTUE IN HARROGATE WATERS. *Dr.* JAMES INGLIS observed that goitre was common in the country around Harrogate but rare in the town itself. He ascribed this immunity to the presence of iodine and bromine in the Harrogate water.
1846	IODINE DEFICIENCY THEORY. Arguing from the successful therapeutic results obtained by CONNET (*q.v.* 1819) with iodine, J. L. PRÉVOST (1790-1850) of Geneva, in collaboration with his Italian colleague A. C. MAFFONI (1806-1878) first suggested that a deficiency of this very element in drinking water is the cause of simple endemic goitre.
1850 to 1876	FIRST CHEMICAL PROOF, by the French analyst G. A. CHATIN (1813-1901), that deficiency of iodine in air, water, soil and food is the primary cause of endemic goitre.
1895	DISCOVERY IN THYROID. Fundamental discovery that iodine is an invariable constituent of the normal thyroid gland was made by E. A. G. BAUMANN of Freiburg.
1913 to 1919	ISOLATION OF THYROID IODINE HORMONE. The iodine-containing principle of the thyroid gland was first isolated in crystalline form by E. C. KENDALL, M.D., Rochester, who showed it to contain 65 per cent of iodine, and named it *Thyroxine*.
1925 to 1930	SYNTHESIS OF THYROXINE. CHARLES R. HARINGTON, F.R.S., determined the chemical constitution of *Thyroxine* to be tetra-iodothyronine, devised means for its artificial synthesis, and pointed out the principal chemical features responsible for its specific physiological activity.
1952	TRIIODOTHYRONINE. Discovery by JACK GROSS and ROSALIND PITT-RIVERS that *Triiodothyronine* is present in the thyroid gland and forms a proportion of the circulating hormone, marks a new forward step towards eventual elucidation of precise chemical and physiological purpose of iodine within the animal body.

* *First President of the Royal Society of Medicine after its incorporation by Royal Charter in* 1834.

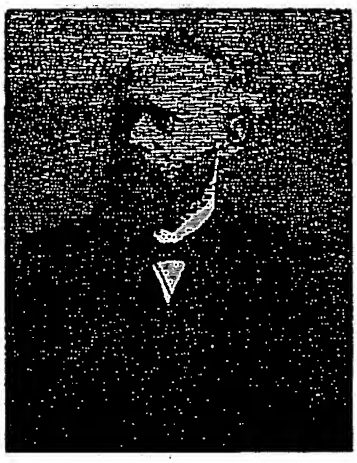


1. J. G. A. Lugol (1786-1851) originator of the aqueous solution of iodine (with KI) for treating scrofula.

2. Humphry Davy (1778-1829) wrote the first account of iodine in English.



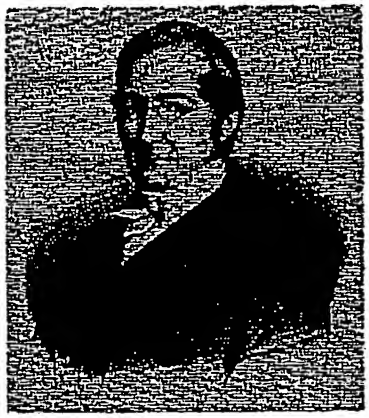
3. Gaspard Adolphe Chatin (1813-1901) first to show that deficiency of iodine in air, water and soil is associated with the occurrence of endemic goitre.



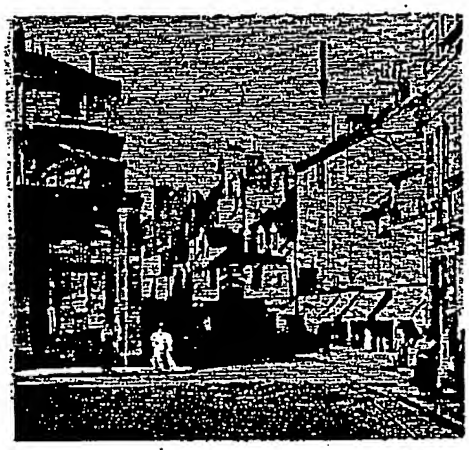
4. Eugen Baumann (1846-1896) discovered that iodine is an invariable constituent of the normal thyroid gland.

5. Thomas Wharton (1614-1673) described and named the thyroid gland in 1656.

6. Jean-François Coindet (1774-1834) introduced tincture of iodine as a goitre specific in 1819.



7. J. L. Gay-Lussac (1778-1850) proved iodine to be an element and named it.



8. The house in Dijon where Bernard Courtois was born.

(Photographs 1, 5, 7 reproduced by courtesy of the Wellcome Historical Medical Museum, London)

MATERIA MEDICA

1821 MAGENDIE'S FORMULARY. Iodine included for the first time in a prescribers' manual—the *Formulary* of FRANÇOIS MAGENDIE (1783-1855).

1821 IODINE TINCTURE INTRODUCED TO ENGLAND. *Sir* ANDREW HALLIDAY, M.D. (1781-1839) first to make detailed recommendations to the medical profession in England of the therapeutic uses and advantages of iodine preparations (especially the tincture) for diseases other than goitre.

1822 IODOFORM discovered by the French chemist, SÉRULLAS. Arguments for and against its medical efficacy continued throughout the 19th Century.

1829 INHALATIONS OF IODINE VAPOUR first recommended by *Sir* JAMES MURRAY, M.D. (1788-1871) for consumption, croup, catarrh, asthma and other respiratory diseases.

1829 LUGOL'S SOLUTION. J. G. A. LUGOL (1786-1851), physician in the Hospital of St. Louis, Paris, introduced the aqueous solution of iodine (with KI) for the treatment of "les maladies scrofuleuses". Wrote three memoirs on the subject.

1839 WOUNDS. First specific reference to the use of tincture of iodine in *wounds* made by JOHN DAVIES, surgeon, Hertford.

1851 POLAROID. WILLIAM B. HERAPATH, M.D. (1796-1868), professor of chemistry at Bristol Medical School, discovered that iodoquinine sulphate ("Herapathite") has light-polarizing properties. Forerunner of the modern *Polaroid*.

1862 BATTLEWOUNDS. Earliest recorded account of iodine tincture applied to wounds *sustained in battle* (American Civil War).

1864 IODINE finds a place in the 1st British Pharmacopoeia as *Linimentum Iodi* and *Tinctura Iodi*.

1865 ANTISEPTIC. A. A. BOINET, French surgeon, published his classic, *Iodothérapie*, in which he describes iodine as "un antiseptique, un désinfectant précieux."

1873 GERMICIDE. C. J. DAVAINE, French bacteriologist; first *knowingly* to employ iodine tincture as an agent for the destruction of infective organisms (anthrax).

1881 ROBERT KOCH (1843-1910). By common consent the greatest pure bacteriologist of his era. First to emphasize the germicidal potency of *aqueous* iodine solution.

1902 CATGUT STERILIZATION. M. Claudius, Danish bacteriologist, inaugurated a new safety era in surgery by recommending aqueous solution of iodine for the sterilization of surgical sutures. Aqueous iodine remains today the only effective *chemical* disinfectant of raw catgut.

1908 SKIN DISINFECTION. German surgeon, A. Grossich, was first to define and apply a rigid technique—known as the *Grossich technique*—for pre-operative skin sterilization by means of iodine tincture.

1910 "BRYANT'S SHERRY." Synonym for tincture of iodine. London surgeon Thomas Bryant* (1828-1914) wrote in 1910: "After 40 years experience I am pleased to express an opinion that it [iodine] is without doubt the best antiseptic that surgeons of the present day possess."

1916 "BIPP". Rutherford Morison, F.R.C.S. (1853-1939), famous Newcastle surgeon, originated *Bipp* (bismuth iodoform paraffin paste). "In *Bipp* we have discovered an antidote to true sepsis, and can leave dirty wounds undressed for a whole month."

1919 TRANSITION. Alexander Fleming (1881-1955) by exhaustive experiments at St. Mary's Hospital, London, in collaboration with *Sir* Almroth Wright, proved conclusively that disinfection of an already infected wound by any then known chemical disinfectant (iodine among them) is not possible except at a strength destructive to body tissues. Ten years later (1929) *Sir* Alexander Fleming, F.R.S., discovered the first antibiotic, *Penicillin*, which virtually eclipsed purely chemical methods of disinfecting blood and body tissues.

1921 CONTRAST MEDIA. Advent of iodized poppyseed oil, first radio-opaque iodine contrast medium. Today (1961) iodo-contrast media account for more than 150 tons of iodine annually.

1939 END OF AN ERA. Last and perhaps greatest protagonist of direct energetic iodine disinfection of wounds was *Sir* Leonard Erskine Hill, F.R.S. (1866-1952) director of research, St. John Clinic and Institute of Physical Medicine, London. Convinced iodine veteran of World War I, he returned to its defence on outbreak of World War II, extolling virtues of intensive iodine disinfection of wounds "in all their depths and ramifications". Views not accepted by the moderns. With him died finally the 1914-1918 war faith in the iodine "first field dressing."

1949 IODOPHORS. Iodine tamed. Herman Shelanski, M.D., of Philadelphia discovered that polyvinyl pyrrolidone and various surfactants solubilize iodine to form highly active complexes which do not sting or stain.

* *President of the Royal Society of Medicine*, 1898.

WORLD CONSUMPTION OF IODINE BY SOURCE OF PRODUCTION

YEAR	EPISODE	SOURCE	WORLD CONSUMPTION OF IODINE (10-year averages, excluding USSR.)				
			DECADE	SEAWEED	CALICHE	WATERS	TOTAL
				(METRIC TONS)			
1811	BERNARD COURTOIS: discovers Iodine in the ashes of	Seaweed					
1820-40	FRANCE, sole world producer	Seaweed					
1840	HAYES discovers Iodine in Chilean	Caliche	1840/49	31			31
1841	SCOTLAND begins production	Seaweed					
1855	JACQUELAIN: 1st extraction from Chilean	Caliche	1850/59	65			65
1860	IRELAND begins production	Seaweed	1860/69	90	1		91
1868	CHILE: first export of Iodine	Caliche					
1879	NORWAY begins production	Seaweed	1870/79	92	26		118
1888	JAPAN begins production	Seaweed	1880/89	72	164		236
1892	JAVA begins production	Waters	1890/99	100	345	2	447
1900	SOUTH AFRICAN WAR (1899-02)		1900/09	186	346	11	543
1914	WORLD WAR I (1914-18)		1910/19	200	627	31	858
1926	U.S.A. begins production	Waters	1920/29	177	626	53	856
1927	ITALY begins production	Waters					
1933	JAPAN begins production	Waters	1930/39	146	632	229	1007
1934	IRELAND and NORWAY cease production from Seaweed						
1936	SCOTLAND ceases production from Seaweed						
1938	JAPAN ceases production from Seaweed						
1939	WORLD WAR II (1939-1945)						
1940	JAVA ceases production from Waters		1940/49	42	889	348	1279
1950-59	CHILE, JAPAN, U.S.A., ITALY, sole	Caliche &	1950/59		1068	735	1803
1960-	producers today (excluding USSR.)	Waters	1960		~1200	~800	>2000

Table 2

Printed in England by Cob Litho.Ltd.

live up to preconceived ideas were exposed, at St Mary's Hospital, in classic experiments by Sir Almroth Wright and Alexander Fleming (later Sir Alexander Fleming of penicillin fame). And, as a result, iodine was withdrawn from the 'first field dressing' and from all official procedures by which wounds were treated in the Navy, Army and Air Force.

On the outbreak of the Second World War in 1939, one or two redoubtable diehards – I cannot but admire their tenacity – sprang to a renewed defence of iodine for swabbing wounds 'in all their depths and ramifications'. These efforts, however, did nothing to influence the contemporary outlook. All they did was to mark the end of what I call the energetic era of wound management.

To-day, there are only three major disinfectant roles for which iodine is fitted – and for which it has no equal. These are: rapid disinfection of unwashed intact skin; the sterilization of surgical catgut (iodine is the only chemical that will do this infallibly); and, thirdly, the emergency sterilization of infected drinking water.

Contrast Media

An entirely new chapter in the medical fortunes of iodine opened with the discovery of X-rays in 1895 – a development that put a new diagnostic aid into the hands of physicians, and brought undoubted advantage to iodine producers and pharmaceutical manufacturers alike.

Soon after their discovery, the suggestion was made that substances through which X-rays cannot pass might be introduced into the body to provide contrast shadows and so make organs and tissues visible on a radiographic film.

Iodine is a substance endowed with the power to stop X-rays. Moreover, it has other attributes desirable in the make-up of contrast media as they are called. The year 1921 saw the first contrast medium in use – an iodized oil named Lipiodol. By introducing a little of this into the lungs and air cavities, and then applying the X-ray, a shadow picture of the bronchial tree is obtained from which experts can readily determine whether all is well or something wrong.

Brain, arteries, the veins, kidneys, liver, gall-bladder, the reproductive organs, and almost every other bodily structure may be examined in this way. To-day, about 30 basic iodo-contrast media are available. But by the requirements of pharmacy firms, these same 30 are sold under something like 200 different proprietary brands and designations.

Chemotherapy

The turn of the century also saw the birth of a new chemistry – biochemistry – which brought with it the gradual ascendancy of rational over empirical modes of treatment. Broadly speaking, chemotherapy, or replacement therapy, is the intelligent use against disease of these very drugs, extracts and principles which the living body continually manufactures within itself to keep itself alive, and whose deficiency or absence through some mechanical breakdown gives rise to disease. For example: insulin *versus* diabetes; liver extract *versus* anæmia; cortisone *versus* adrenal deficiency and a whole lot else; and, as we have already discussed, thyroid extract and thyroxine *versus* thyroid abnormality.

This broadening and deepening outlook on therapeutics is clearly reflected in the changing composition of official and non-official pharmacopœias. The old, and to us perhaps rather crude, materia medica – predominantly inorganic in kind and content – is giving place to a vocabulary of finer pharmacological texture – largely organic.

Not unnaturally, iodine has shared in this progressive movement, and Table I (*see* insert) shows how the British Pharmacopœia of modern days has taken on an entirely new look so far as iodine is concerned. Since 1864, there have been nine 'B.P.s' plus an addendum in 1960. Horizontal lines in colour mark the stages at which various iodine preparations were introduced, and the length of their survival as accepted therapeutic agents.

If one separates the inorganic monographs from the organic, that is, the simple from the complex, the upper and lower halves of the table – and take, for example, the 3rd 'B.P.' (1885) – one finds in it 13 inorganic iodine preparations and only one organic item, iodoform. Seventy-five years later (1960) only 6 basic inorganic, and 12 organic items are seen – an almost complete reversal.

On top, in red, are the parent substance, the strong and the weak tinctures, and potassium iodide – all with a record of one hundred years. Lugol's solution (5th on the list, top half) had a first innings of twenty years, was removed for thirty years, but was reinstated in 1948, and is still 'not out'.

Below, the organic members of the team are all comparative newcomers, and, as we have noted, are used for therapeutic purposes wholly different from those customary in former times. The switch in usage has been from skin disease,

venereal disease, and general tonic purposes (top half) to contrast media, thyroid therapy and the control of specific tropical diseases by organics such as chiniofon and iodo-hydroxyquinoline (lower half).

So much for the pharmacy and medicine of iodine.

Sources and Consumption

To occupy the time left at my disposal I would like to make a very brief historical note on the commercial sources of iodine, and upon world consumption as it was in the beginning and as it is today.

Table II (*see* insert) shows the average annual world consumption of iodine, decade by decade, for one hundred and twenty years from 1840 to 1960; and the amounts contributed to this consumption by the three great producing sources – seaweed (in green), Chilean *caliche* (in red) and underground waters (in blue). The totals are in metric tons; no data exist prior to 1840.

Seaweed, in which iodine was discovered, remained the only commercial source until Chile entered the field in 1868. Chilean iodine is obtained as a by-product during the elaboration of Chilean nitrate of soda from the raw material called *caliche* which occurs in vast natural deposits in Northern Chile.

From 1868 onwards, the Chilean share of the world iodine market gradually matured and overtook the seaweed share around 1880/1890. As a consequence, the seaweed contribution to world needs – provided mainly by France, Scotland and Japan, and with a zenith of 200 tons per annum in the decade 1910/1919 – gradually declined and fell to zero at the end of the Second World War. No seaweed iodine is produced to-day anywhere in the world, unless it be in Russia, which is doubtful.

In 1892 a new and powerful impact struck iodine commerce when workable quantities of the element were found in certain underground waters – mineral springs and natural brines, particularly in oil-field areas.

Java (in 1892) was the parent of this enterprise; California followed in 1926; Italy in 1927; and Japan in 1933. With the final elimination of all seaweed production in 1945, world needs are now supplied entirely from Chilean *caliche* and from underground waters in Japan, the United States, and Italy. To-day, world consumption lies between 2,000 and 2,500 tons – four times more than at the beginning of this century and twenty times more than one hundred years ago.

The figure mentioned excludes Russia and covers all fields of usage. Broadly speaking, 50% of the total is used for medical purposes; 30% goes into technical industry; and the remaining 20% into veterinary medicine and agriculture. This means that 1,000 to 1,200 tons are absorbed every year into pharmaceutical and medical use; and of this quantity 60% is converted and used in the form of potassium iodide.

These figures are near approximations. It is exceedingly difficult to obtain reliable statistics on the end-usage of iodine at any given moment of time. All sorts of unexpected factors and circumstances may intervene to upset one's calculations as, for example, when during the last war an exceptionally heavy and persistent demand arose in certain neutral countries for an iodine preparation called Entero-Vioform, sought ostensibly for the treatment of parasitic disease. In fact, this was none other than an attempt to smuggle iodine into Germany where supplies of crude material were non-existent. A more interesting irregularity occurred in the United States around the year 1927 when the iodine industry was surprised to notice an enormously increased demand for the alcoholic tincture of iodine. This was the era of prohibition in America, and it soon became apparent that the unprecedented sales of alcoholic iodine tincture were not for legitimate medical use, but simply to recover alcohol for 'bootlegging'.

Special legislation was introduced to control this nefarious trade, but not before 50 tons of iodine had been illegally diverted – representing approximately one and a quarter million litres of absolute alcohol.

What of the future? Who can tell how the reputations of iodine to-day will stand one hundred years hence? To venture an opinion, based, as it can only be, on the events of the past, I can only say that the process of research, revaluation, reappraisal, refinement, will go on, and that in the year 2061 some iodine merits that enjoy contemporary favour will plainly appear ephemeral, while new merits as yet hidden from us will assuredly have declared themselves. We may safely leave it to our successors to share the surprises that time has yet in store.

The 8-page insert accompanying this paper contains Tables I and II referred to in the text, a list of important dates in the medical history of iodine, and photographs of several of the great personalities who have helped to make the iodine story.

ENCLOSURE 1A

PVP-Iodine: History, Toxicity and Therapeutic Uses

HERMAN A. SHELANSKI, Ph.D.†
AND
MORRIS V. SHELANSKI, M.D., C.M.*
PHILADELPHIA, PENNSYLVANIA

POLYVINYLPYRROLIDONE (povidone)[1]** meets the criteria for a successful expander of blood volume. It is one of the final products of a series of reactions starting with acetylene and formaldehyde and is similar in configuration to the breakdown products of hemin except that there are many more pyrrole rings in PVP, and the linkage between the rings is by means of vinyl groups bridging the nitrogens rather than methene bridges joining the adjacent carbons.

Physical Properties.—Povidone (PVP) is a nearly white powder, readily soluble in water and many organic solvents. It is stable to heating in air at 100 C. for at least sixteen hours. The powder is hygroscopic, but otherwise stable, and can be stored indefinitely.

Physiologic Properties.—Povidone resembles the proteins of natural plasma in its capacity for binding water and adsorbing other substances. These properties have been attributed to the presence of the amido (-CO-N-) groups. Because of its high collodial osmotic pressure, PVP (2.5 per cent, later 3.5 per cent) in physiological solution of sodium chloride (periston) was used extensively by the Germans in the treatment of shock.[2] PVP, after intravenous administration, was shown by Weese and Hecht[3] to assume the carrier functions of the plasma proteins. Bennhold, Schubert and others[4] demonstrated by cataphoretic and diffusion experiments that, in addition to its physical resemblance to plasma proteins, PVP shows a biologic resemblance by adsorbing physiologic and nonphysiologic products, including bilirubin, atabrine, Vitamins B-1, C, and P and numerous dyes, as well as the toxins of diphtheria, tetanus and botulinus. In addition, intravenously injected PVP exhibits another characteristic of plasma protein. This is the so-called embathic effect, which is the ability to render diffusible coarsely dispersed dyes such as congo red.[5]

Pharmacologic Properties.—PVP displays a retarding and potentiating effect on a large number of parenterally administered drugs. This effect was first observed in Germany by Duttmann,[6] who used it to prolong the action of pontocaine in peridural anesthesia. Further advances in the utilization of this characteristic have been made in France, England and the United States, especially in intramuscular antibiotic preparations.

Schubert and his associates have claimed that PVP detoxifies *in vivo* certain dyes and certain toxins, including those of diphtheria, botulinus and tetanus. They postulated a mechanism in which the PVP protects the liver by diverting the toxins to the kidney and by increasing the rate of excretion through that organ into the urine by virtue of its embathic effect.[7]

Discovery of PVP-Iodine.—In view of

†Deceased.
*Research Fellow, Philadelphia General Hospital; Director of the Industrial Toxicological Laboratories, Philadelphia, Pa.
**Povidone is distributed by the Iodine Pharmacal Corp., Denver, Delaware.
Submitted for publication May 9, 1956.

the detoxifying properties of PVP, Shelanski, Cantor and Shelanski[8] studied its effects upon inorganic toxic materials. One of the first of such materials studied was iodine in Lugol's solution and as a tincture.

In preliminary studies of acute oral toxicity on albino rats, Lugol's solution and tincture of iodine in combination with PVP were shown to be less toxic than was either solution alone. Altered physical properties were produced by the presence of PVP. Most obvious was the altered color of the solution. Instead of the red or purplish color expected, a rich mahogany color was obtained. Coupled with this was the observation that the vapor pressure of the iodine was considerably reduced. It was logical to assume that the iodine was undergoing some alteration in the presence of the PVP. In attempting to titrate back the iodine, it was discovered that not all the iodine could be recovered. Repeated extractions with chloroform were unsuccessful in retrieving the missing portion of iodine. Further additions of Lugol's solution or tincture to the iodine-containing PVP, however, resulted in no similar loss of iodine. At this point, Shelanski and Shelanski theorized that the lost iodine was bound to the PVP in the form of organically bound iodide, which should act to stabilize iodine in the same manner as potassium iodide in Lugol's solution. They prepared an aqueous solution of iodine combined with PVP without the aid of the usual solvents or solubilizing substances. Thus, it was shown that PVP itself acts as a carrier of iodine in water solutions. It was later shown that iodine vapors are directly soluble in PVP powder.

Chemical Nature of PVP-Iodine. — It was shown that PVP acts in a manner similar to plasma protein in combining with iodine. A small fraction (less than 1 per cent) of the iodine is converted directly into inorganic iodide. The reaction occurs at the ends of the PVP molecules, where an acid environment prevails. The amount of inorganic iodide formed is determined by the number of free end groups and thereby by the molecular weight of any given PVP sample. About 30 per cent of the iodine is converted into organic iodide (corresponding to protein-bound iodine). The remainder of the iodine exists as free elemental iodine. The free iodine is made soluble by the organic iodide. There is still another factor, however, which is responsible for the solubility of the iodine. In Lugol's solution, 2 parts of potassium iodide are required to make 1 part of iodide soluble. In PVP-iodine the proportions are reversed; about 30 per cent of the iodine is converted to iodide, and 70 per cent remains free. The reason for this phenomenon is not completely clear, but it appears that a two-phase system exists. The free iodine actually is in solution in the PVP iodide. The PVP-iodide-iodine complex in its entirety is in solution in the water.

Effectiveness Against Microorganisms. —Cantor and Shelanski proceeded with the *in vitro* assay of PVP-iodine. It was demonstrated that the iodine in PVP-iodine was available to perform the same functions as iodine in tincture or Lugol's solution, namely, its bacteriocidal, viricidal and protozoocidal capabilities had not been decreased. With use of the capacity method[8] developed by Cantor and Shelanski, it was shown that the capabilities of iodine had been prolonged.

Toxicity of PVP-Iodine.—Concurrently these authors, investigating the toxicity of PVP-iodine in mammals, noted that iodine in this form was less toxic than iodine in Lugol's solution or tincture.

The toxicity studies were performed with PVP-iodine solutions containing 1 to 5 per cent iodine and 2 to 10 per cent PVP in distilled water.

Acute Oral Toxicity Study. — Twelve groups of animals, each containing 10 albino rats of the Yale-Sherman-Wistar strain, were used in testing Lugol's solution containing 2.5 per cent free iodine, and an equal amount was used in studying a PVP-iodine solution containing 2.5 per cent free iodine. These rats were observed for two weeks to make sure that they were normal. Litter mates were dispersed throughout all groups of both studies. From twenty-four hours prior to dosing, the animals were given water only. Dosing was performed by means of a rubber catheter introduced via the mouth directly into the stomach. Doses were accurately measured by means of a calibrated syringe. Survival counts were taken daily for two weeks after administration of the iodine solutions. The LD_{50} results were as follows:

Lugol's solution—400 mg. of free iodine per kilogram.

PVP-iodine—1,300 mg. of free iodine per kilogram.

This shows that PVP-iodine is more than three times as safe to use as is Lugol's solution containing an equal amount of free iodine.

Another group of investigators who performed this study obtained an LD_{50} of 107.5 mg. of free iodine per kilogram for Lugol's solution and 962.5 mg. of free iodine per kilogram for the PVP-iodine solution. In these results there is a 9 to 1 ratio with regard to the safety of use of the PVP-iodine solution.

Percutaneous Studies on Intact Skin.— Twenty-five rabbits were prepared by clipping the hair from the back to expose a large area of skin. PVP-iodine, containing 10 per cent PVP and 2 per cent iodine, was applied over an area of 1 square inch on one side of the animal. On the other side a tincture of iodine solution containing 2 per cent free iodine was applied in a similar manner and used as a control. The contact sites were covered with lintine discs, which in turn were covered with waxed paper and held in place with adhesive tape. The patches were scheduled to be left on for ninety-six hours. Within twenty-four hours, however, severe erythema and edema developed in the sites of contact with tincture of iodine, necessitating removal of these patches. No such reactions developed with the PVP-iodine, even though the patches remained in place for the scheduled ninety-six hours. After a two-week period of recuperation the patches were reapplied in an identical manner. Again the tincture of iodine patches had to be removed prematurely because of severe reactions. After forty-eight hours the PVP-iodine patches had produced no cutaneous reactions.

With the same solutions, 200 human subjects were exposed to a similar patch test. As in the case of the rabbits, the tincture of iodine patches had to be removed prematurely (within twenty-four hours) because of complaints caused by the severe cutaneous reactions. The PVP-iodine patches produced no such reactions, even after ninety-six hours of contact, when they were removed.

After two weeks the patches were reapplied in the same manner, and again the reactions to the tincture of iodine were severe within twenty-four hours. No such reactions occurred with the PVP-iodine, even after forty-eight hours.

One hundred volunteer persons were subjected to the repeated insult patch test method of Shelanski and Shelanski.[9] The same solutions of tincture of iodine and PVP-iodine were tested. The patches were scheduled for twenty-four hour contact, every other day, with 15 applications. The tincture of iodine patches were discontinued after the first application because of severe reactions, even though the patches had been removed within twenty-four hours. No complaints or reactions were

caused by the PVP-iodine patches throughout the 15 applications. Even after a challenge application, which was carried out after three weeks and consisted of contact for forty-eight hours, the PVP-iodine elicited no subjective complaints and no visible reactions.

Percutaneous Studies on Damaged Skin.—All the aforedescribed studies were repeated on those rabbits and human subjects in which the skin of the contact sites was purposely damaged by abrasion with coarse sandpaper. In all cases the results were essentially the same. In addition, it was observed that no infections occurred; all the abrasions were healed or in the process of healing when the PVP-iodine patches were removed.

Mucous Membrane Exposures.—Twenty-five rabbits were used to determine and compare the irritating effects of a PVP-iodine solution and Lugol's solution when instilled into the eyes. The PVP-iodine solution contained 10 per cent PVP and 1 per cent iodine. The Lugol's solution contained an equal amount of free iodine. One-half cc. of the PVP-iodine was instilled into the left eye of each of the rabbits and the same amount of the Lugol's solution was instilled into the right eye as a control. The eyes were observed daily for two weeks. The PVP-iodine produced a slight reddening of the conjunctiva which disappeared within two or three days. The Lugol's solution produced severe erythema, edema and progressive corneal damage, which did not clear up within the two-week period of observation.

The effects of repeated daily instillations of the two solutions were studied in a group of 25 guinea pigs and 25 rabbits. It was planned to instill 0.1 cc. of PVP-iodine daily into the left eye of each animal and 0.1 cc. of Lugol's solution into the right eye. The reactions were so severe with the latter that the instillation of Lugol's solution was discontinued after three days. The PVP-iodine, however, produced transitory erythema, which cleared within a few hours. Instillations with the PVP-iodine were continued for the entire fifteen-day period without any significant damage. Two weeks after the fifteenth instillation a challenge instillation was performed with PVP-iodine. The results were identical with those observed before.

A group of 25 human volunteers was used in the next study. The mucous membranes of the throat, including the tonsillar fossae, the palate, the uvula, and the posterior pharyngeal wall were swabbed with approximately 2 cc. of PVP-iodine. The reactions ranged from a stinging sensation, which abated in ten to twenty minutes, to no sensation but a "bad taste." The subjects were examined for reactions every half hour for the first two hours and every hour thereafter for the next four hours. There was a slight reddening of the mucous membranes in about 60 per cent. Forty per cent showed no reaction. The reddening persisted for two hours or less. These studies were repeated daily, fifteen applications being tested on 10 patients, 5 of whom showed no reactions at all to the PVP-iodine and 5 of whom showed reddening after initial application. In none of these patients was there any aggravation of the initial reaction to the PVP-iodine. After two weeks of rest these 10 patients were again treated in the same manner, and the reactions noted were similar to those previously noted.

Effect on Iodine-Sensitive Humans.—Three known iodine-sensitive volunteer subjects were tested with patches of PVP-iodine and Lugol's solution. The skin in contact with the Lugol's solution erupted with a severe reaction, typical of the sensitization-type reactions. None of the patients showed any reaction to the PVP-iodine.

Therapeutic Evaluation of PVP-Iodine.—Except when otherwise noted, PVP-iodine solutions containing 2 per cent free iodine and 10 per cent PVP were used. Two series of patients were treated.* The first series consisted of patients with surface infections, both bacterial and mycotic, of the skin and mucosa. The second series of patients had wounds of the skin or mucosa, and the group included those on whom operations were performed. More than 300 patients were treated.

Series 1: Polyvinylpyrrolidone-Iodine for Treatment of Surface Infection.—A total of 108 representative patients with bacterial and mycotic infections of the skin and mucosa were treated with the unaltered PVP-iodine solution, consisting of varying proportions of iodine and PVP in water. All showed marked improvement within eighteen hours, and in 100, or 94.3 per cent, the condition cleared up within thirty-six hours. In detail the series consisted of 62 cases of bacterial infections of the skin, 23 of sore throat, 12 of mycotic infections of the toes, 6 mycotic infections of the hands, 2 of mycotic infections of the ears and 3 of mycotic infections of the perineal region. Typical case histories are as follows:

CASE 1.—A 24-year-old white man had a history of recurrent sycosis barbae, which had responded slowly to treatment. PVP-iodine was applied to the chin and cheeks without producing any additional irritation of the lesions. Within four hours the patient noted relief from the irritation produced by the infection. Within eighteen hours the lesions had become noticeably drier. PVP-iodine was applied a second and third time after twenty-four hours and forty-eight hours respectively. Complete recovery was noted on the third day after the treatment had been started. During this time the patient was not allowed to shave or wash his face.

CASE 2.—A 32-year-old white man had an infected wound on the dorsal aspect of the right little finger. The wound was purulent, ulcerated and inflamed. Enlarged lymph nodes were present in the epitrochlear and axillary regions. The temperature was 99 F. The wound was cleansed; PVP-iodine was applied, and the wound was dressed. No increased irritation was noted by the patient. After twenty-four hours the temperature was normal. Lymphadenopathy had diminished. The wound itself was dry and clean, and epithelization was in progress.

CASE 3.—A 38-year-old white man had had bilateral mycotic infection of the ears for several months, and all previous treatment had failed. PVP-iodine was instilled into both ears, and a superficial cotton plug was used to close the canals. Relief from itching was noted in one hour. After twenty-four hours reexamination showed desquamation of the epithelium and a decrease in the amount of discharge. The drum appeared almost normal except for some discharge on the surface. A second application of PVP-iodine was made at this time. On the next day, examination revealed that the left ear was completely dry. The right ear still had some discharge, but there was no itching. PVP-iodine was again applied in both ears. Four days after the start of the treatment both ears appeared completely normal. The patient was relieved of itching and has continued well.

CASE 4.—A 21-year-old white man had had gross mycotic infection of the right foot for the past eight years. No previous treatment had produced any relief. On first examination the dorsal aspect of the foot was covered with weeping sores. The patient stated that the itch was so unbearable that he had to scratch almost continuously. PVP-iodine was applied to the lesions, and the foot was bandaged. Twenty-four hours later, reexamination showed a marked decrease in the discharge. The patient volunteered the information that he was able to sleep peacefully, without interruption, for the first time in many years. Daily applications of PVP-iodine were continued for one week. Marked improvement was noted, although the lesions had not completely healed. Tincture of 30 per cent PVP-iodine was prepared and given to the patient for self-treatment. At the time of writing the lesions are minimal and dry. Since they are still present, however, we consider this case a failure.

CASE 5.—A 30-year-old white man complained of sore throat of one week's duration. He had undergone intensive treatment by his family physician, without relief. The temper-

*These patients were treated at the Philadelphia General Hospital and in the Medical Department of General Motors Corporation, Wilmington, Delaware.

ature was 99.9 F., and the cervical lymph glands were swollen and tender. The entire pharynx was markedly injected. The patient's throat was swabbed with PVP-iodine. After twenty minutes the pain had completely abated. The patient could swallow without distress for the first time since his illness began. On the next day the temperature was normal and all complaints had disappeared.

CASE 6.—A 25-year-old white woman complained chiefly of sore throat and earache of two days' duration. The temperature was 100.1 F. Examination revealed marked injection of the right side of the pharynx and edema of the opening of the eustachian tube. PVP-iodine was applied to the pharynx by means of a swab. After thirty minutes the patient's complaints of pain had diminished. Reexamination showed that the eustachian tube was now open. The temperature had subsided to normal in four hours.

These cases are typical of the entire series. Of the 6 cases in which treatment was considered to have failed, there were long-standing fungous infections in 3. These, although considerably improved by PVP-iodine, did not clear up completely or recurred. In the other 3 cases there were bacterial infections treated late in their course, so that generalized therapy was also deemed necessary. The improvement in these cases could not be attributed wholly to local use of PVP-iodine, since penicillin was also given intramuscularly.

Series 2: PVP-Iodine Treatment of Wounds and Rise in Preoperative Cases.—Sixty-seven wounds of various sorts, from superficial skin cuts to deep muscular and tendon lacerations, were treated. In addition, in 34 surgical cases PVP-iodine was used as a cutaneous and incisional germicide. Typical cases are as follows:

CASE 7.—A 28-year-old white man sustained a deep laceration of the left palm. The skin over the thenar eminence was completely undercut for approximately 2 cm., the tendons and subcutaneous structures being left exposed. The patient's hand was completely covered with grease and dirt, which was impossible to remove with soap and water. The entire hand was prepared with PVP-iodine. The patient complained of some burning when the PVP-iodine was applied directly into the deep laceration. For comparison, a small amount of alcohol was placed in one corner of the cut. The patient stated emphatically that the pain had increased considerably with the alcohol. The closure of the wound required 4 subcutaneous absorbable sutures, and 12 wire sutures were used to close the skin. In spite of the massed contamination present when the laceration occurred, recovery was uneventful, without any infection. The patient did show an elevation of temperature to a maximum of 100 F. twenty-four hours after the injury.

CASE 8.—A 40-year-old white man was lifting a heavy box, which dropped on his right middle finger and amputated the distal phalanx. When first seen, the patient was bleeding profusely; his hands were completely covered with grease and dirt, which was impossible to remove with soap and water or thinner. After the bleeding had been controlled the entire hand was prepared with PVP-iodine. The patient complained of burning caused by the PVP-iodine on the raw surface of the finger. A drop of tincture of a mercurial was placed on the raw surface for comparison. The patient emphatically stated that the pain was much less intense with the PVP-iodine. His foreman in the meantime had retrieved the amputated fragment of finger. It was decided to attempt to graft the fragment, even though it had been lying on the floor for about fifteen minutes. The fragment was bathed for five minutes in a warm PVP-iodine solution. It was reapproximated by anchoring the bone ends by means of wire sutures and the skin ends with 10 wire sutures and a wire stay suture. The finger was splinted and bandaged. After five days the dressing was removed and the wound cleansed. It was without infection and in the process of knitting. The sutures were removed on the tenth day. At this time the distal third of the fragment had become dark and shriveled. The proximal two-thirds was somewhat swollen but viable. The shriveled fragment was removed and the skin edges reapproximated, with PVP-iodine used for antisepsis. Recovery was uneventful.

CASE 9.—A 22-year-old white man had an infected sebaceous cyst behind the left ear. The skin was prepared with PVP-iodine. The cyst was incised, and purulent cheesy material was removed. A small drain soaked in PVP-

iodine was inserted into the incision, and the wound was dressed. Twenty-four hours later the drain was removed. No evidence of infection was present. No irritation due to the PVP-iodine could be noted.

CASE 10.—A 21-year-old white man had a ganglion on the dorsal aspect of the right wrist, which interfered with his work. The hair was shaved off and the skin prepared with PVP-iodine. A small incision was made over the ganglion; the tissues were separated, and a grape-sized ganglion was noted, arising from the extensor digitorum profundum tendon. The entire wound was coated with PVP-iodine after the ganglion had been excised. The skin was reapproximated with 2 nylon mattress sutures. The sutures were removed in six days. Recovery was uneventful.

CASE 11.—A 40-year-old white man had had the left first upper molar removed and was complaining of pain. Examination revealed a dry socket. The socket was painted with PVP-iodine. Relief was apparent within thirty minutes. A second application of PVP-iodine was made at the end of four hours. No further treatment was necessary.

In addition to these two series, a number of patients with varicose ulcers were treated.

CASE 12.—A white man aged 50 had stasis eczema and an infected varicose ulcer of the right ankle. The duration of the ulcer and the eczema was five years. The patient had undergone saphenous ligation and a variety of local treatment, without effect. PVP-iodine was given him to be applied locally night and morning, in addition to supportive dressings. The patient was seen at weekly intervals, and definite improvement was noted after the first week. Pain was diminished, and the seropurulent discharge cleared, the base of the ulcer showing healthy granulation tissue. After three weeks the ulcer was partially closed; there was no pain, and the stasis eczema subsided. Application of PVP-iodine was continued twice daily, and at the end of six weeks the ulcer was entirely healed, Epithelization being complete, the patient was discharged.

CASE 13.—M. S., a white woman aged 50, had a varicose ulcer on the inner aspect of the left ankle, of four years' duration. Examination revealed marked varicosities. Superficial lesions and one dime-sized ulcer were present on the left ankle. Pain was present and severe. PVP-iodine was applied twice daily, with a supportive bandage. After one week the pain subsided and the ulcer was healing, being now only one-half its former size. The patient was classified as "improved."

CASE 14.—M. S., a white woman aged 65, had a punched-out varicose ulcer the size of a split pea on the anterior aspect of the left leg. There was severe pain, with inflammatory changes. PVP-iodine was applied twice daily, with a supportive dressing. After one week the ulcer was healing and was half its original size. There was no drainage. The pain also had subsided. The patient was classed as "improved."

CASE 15.—F. P., a white man aged 52, had had a dime-sized varicose ulcer on the posterior aspect of the right leg for four weeks. The pain was severe and was associated with stasis edema. PVP-iodine was applied twice daily. After one week the pain had diminished considerably. The ulcer was filling in, and epithelization was beginning. After two weeks there was no pain and the ulcer was almost healed. In addition to PVP-iodine, a supportive dressing was used. This patient also was classed as "improved."

A number of patients with thrush were also treated.

CASE 16.—M. Z., a boy 5 weeks old, was seen in October 1950, with a condition of three weeks' duration, diagnosed as thrush. The area around the mouth was covered with plaques, and the tongue, buccal pouch and pharynx with heavy plaques. The child had been treated with gentian violet (1 per cent), boric acid solution, borax solution and antibiotics. Treatment with gentian violet caused excorciations. The baby would not eat or take the bottle. PVP-iodine was applied three times daily and produced dramatic results in twelve hours. The child began to eat well and was completely cured in forty-eight hours.

CASE 17.—L. F., a woman aged 21, with a condition diagnosed as thrush, was seen in 1951. The lips and tongue were cracked and dry, with areas of plaques. The duration of the ailment was approximately nine months. It had been treated with antibiotics, gentian violet, vitamins and Dobell's solution. PVP-iodine was applied every four hours. An excellent response appeared in twenty-four hours. Within forty-eight hours most of the affected areas were healed.

A report by an oral surgeon who treated

15 patients with conditions clinically diagnosed as thrush and fungous infection under dentures stated that in all cases there was a favorable response to PVP-iodine therapy, usually within a few days.

SUMMARY AND CONCLUSIONS

The chemical, physiologic and pharmaceutical aspects, as well as the uses, of polyvinylpyrrolidone (povidone) have been presented, together with the toxicologic and therapeutic evaluation of PVP-iodine as compared with Lugol's solution and tincture of iodine. These studies show that the toxicity of PVP-iodine is much less than that of iodine in the tincture or in Lugol's solution.

Clinical studies show that PVP-iodine is safe and effective in the treatment of a variety of infections of the skin and mucosa. It has been found effective in the treatment of thrush. With PVP-iodine the germicidal action of the iodine is prolonged and the danger of cutaneous irritation, sensitization, reactions and burns is minimized.

Whereas tincture of iodine presents a hazard on accidental ingestion, PVP-iodine presents no such danger, because it is only from one-fourth to one-ninth as toxic. PVP-iodine presents iodine in a form of low toxicity but of high germicidal action. It has been found valuable in the treatment of a variety of skin and mucosal infections produced by many different types of bacteria.

REFERENCES

1. Hecht, G., and Wesse, H.: Munch. Med. Wchnschr. 90:11-15, 1943. Reppe, W.; Schuster, C., and Hartmann, A. (assigned to I. G. Farben): German Patents 737,663 and 738,753 filed Jan. 17, 1939.

2. Schulz, E.: Deutsch. Med. Wchnschr. 67: 779-784, 7-18, 1941. Tonnis, W.: Neurochirurgie 3:113-161, 1941. Orator, V.: Leitfaden der Feldchirurgie in Bewegungskrieg (pamphlet). Leipzig: J. A. Barth, 1942. Tonnis, W.: Behandlung der Schussverletzungen (pamphlet). Berlin-Munich: J. F. Lehmann, 1942.

3. Wesse, H.: Pharmazie 3:337-340, 1948.

4. Bennhold, H., and Schubert, R.: Ztschr. ges. exper. Med. 113:722-786, 1943. Schubert, R.: Ztschr. ges. exper. Med. 114:634-635, 1945; Artzl. Forsch. 3:425-428, 1949.

5. Bennhold, H.; Ott, H., and Welch, M.: Deutsch. med. Wchnschr. 75:11-15, 1950. Schubert, R., and Wiegandt, E.: Klin. Wchnschr. 24 and 25:273-276, 1947.

6. Duttmann, G.: Zentslbl. f. Chir. 68:530-535, 1941.

7. Schubert, R.: Artzl. Forsch. 3:425-428, 1949. Deutsch. med. Wchnschr. 43 and 44:551-553, 1946. Rev. Gastroenterol. 17:165-179, 1950.

8. Cantor, A., and Shelanski, H. A.: Soap and Sanitary Chemicals, February, 1951. Further Considerations of Germicidal Capacity Testing, pamphlet, 1952.

9. Shelanski, H. A., and Shelanski, M. V.: A New Technique of Human Patch Tests, Proc. Soc. Sect. T.G.A. 19, 1953.

Hilarity and good humor, a breezy cheerfulness, a nature "sloping towards the southern side," as Lowell has it, help enormously both in the study and in the practice of medicine.

The physician needs a clear head and a kind heart.

Avoid wine and women—choose a freckle-faced girl for a wife; they are invariably more amiable.

—Osler